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Ecological Research Series

TRACE ELEMENT RESEARCH USING CONIFEROUS FOREST SOIL/LITTER MICROCOSMS

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U.S. Environmental Protection Agency
Corvallis, Oregon 97330

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TRACE ELEMENT RESEARCH USING CONIFEROUS FOREST SOIL/LITTER MICROCOSMS

by

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FOREWORD

Effective regulatory and enforcement actions by the Environmental Protection Agency would be virtually impossible without sound scientific data on pollutants and their impact on environmental stability and human health. Responsibility for building this data base has been assigned to EPA's Office of Research and Development and its 15 major field installations, one of which is the Corvallis Environmental Research Laboratory (CERL).

The primary mission of the Corvallis Laboratory is research on the effects of environmental pollutants on terrestrial, freshwater, and marine ecosystems; the behavior, effects and control of pollutants in lake systems; and the development of predictive models on the movement of pollutants in the biosphere.

This report encompasses research to detect and understand the effects of trace element pollutants on soil/litter components and on decomposition processes of coniferous forests. It also includes design and development of a microcosm system useful in measuring pollutant perturbations. Data generated in this study will, subsequently, be used in predictive models to determine pollutant impact within the terrestrial ecosystem.

A. F. Bartsch
Director, CERL

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ABSTRACT

Respirometers have been designed, constructed and, to a limited extent, tested to maintain and measure production and/or consumption of biogenic heat and carbon dioxide production and oxygen consumption for extended periods of time in approximately 0.5 l soil and/or litter microcosms.

Using coniferous soil/litter microcosms, the mean coefficient of variation within sets of similar microcosms was 10.7% for the oxygen consumption rate and 3.9% for carbon dioxide production rate.

Microcosm respiratory response, population responses to moisture level (where measured), succession, and salt effects were similar to those observed in the natural world.

Respiration of the decomposer communities in coniferous forest soil/litter microcosms was inhibited by treatment with "real world" salt concentrations of Cd, Se, Zn, Mn, Ni, Cu, Hg, Co, Cr, V , Li, La, Ag, and Pb. These findings support the thesis that the consequence of these ecosystem disruptions might be to reduce primary and secondary production of the dependent populations.

Scale drawings of the microcosm "life-support" system and an outline of procedural details of system maintenance and microcosm preparation are presented.

This report was submitted as partial fulfillment of in-house research under Program Element 1AA006, ROAP 21 ALU, Task 3. It covers the period March, 1974, to November, 1976, and work was completed as of March, 1977.

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I. INTRODUCTION

A. Decomposer Organisms in the Organic Matter Cycle

Decomposition is an essential process in all food webs on this planet and must not be disrupted if biological systems are to remain in temporal equilibrium. The decomposition/primary production system is a cyclic process (Figure 1) in which primary produced organic matter in the form of litter fall, leachates, root exudates and sloughage is remineralized by soil macro- and micro-organisms (the decomposers) to plant nutrients in a series of "shredding" and solubilizing steps (McBrayer et al., 1974; Ausmus and Witkamp, 1974; Gist, 1972). The extent of this process is shown by the estimates that 80 to 90 percent of net primary production in terrestrial ecosystems is ultimately acted on by decomposer organisms (Odum, 1971; Witkamp, 1971; Wittaker, 1970), which along with the primary producers, may make up to 95 percent of the total biomass in deciduous forests (Odum, 1971). It has been estimated that up to 70 percent of the caloric or biomass input to the decomposers is below ground from the roots of the primary producers (McBrayer et al., 1974).

B. Organic Matter Cycle and Tyler's Thesis.

It can be imagined that any interruption in the decomposition portion of this cycle might lead to a decrease in formation of readily utilizable plant nutrients that will subsequently limit plant growth rates (Ausmus and Witkamp, 1974).

It is increasingly apparent that man is disrupting soil decomposition processes, particularly through the addition of trace element pollutants. Examples with anticipated disruptive consequences include fly ash from metal smelters that settles to the ground producing high heavy metal soil concentrations (Ratsch, 1974; Linzon, et al., 1975). Cadmium contamination of phosphate fertilizers (Williams and David, 1973) and miscellaneous trace elements contained in sewage sludge added to agricultural fields (Page, 1974), combustion emission fallout from coal-fired power plants (Klein and Russell, 1973), and roadside accumulations of lead from automobile combustion of tetraethyl lead in gasoline are other examples.

1. Laboratory Evidence.

It is anticipated that many organisms, if not the entire community, in heavy metal contaminated soils are affected by these pollutants. It has been documented that heavy metals as well as certain non metals inhibit bacterial growth (deJong and denDoren, 1971; Salle, 1973; Lamanna and Mallette, 1965),

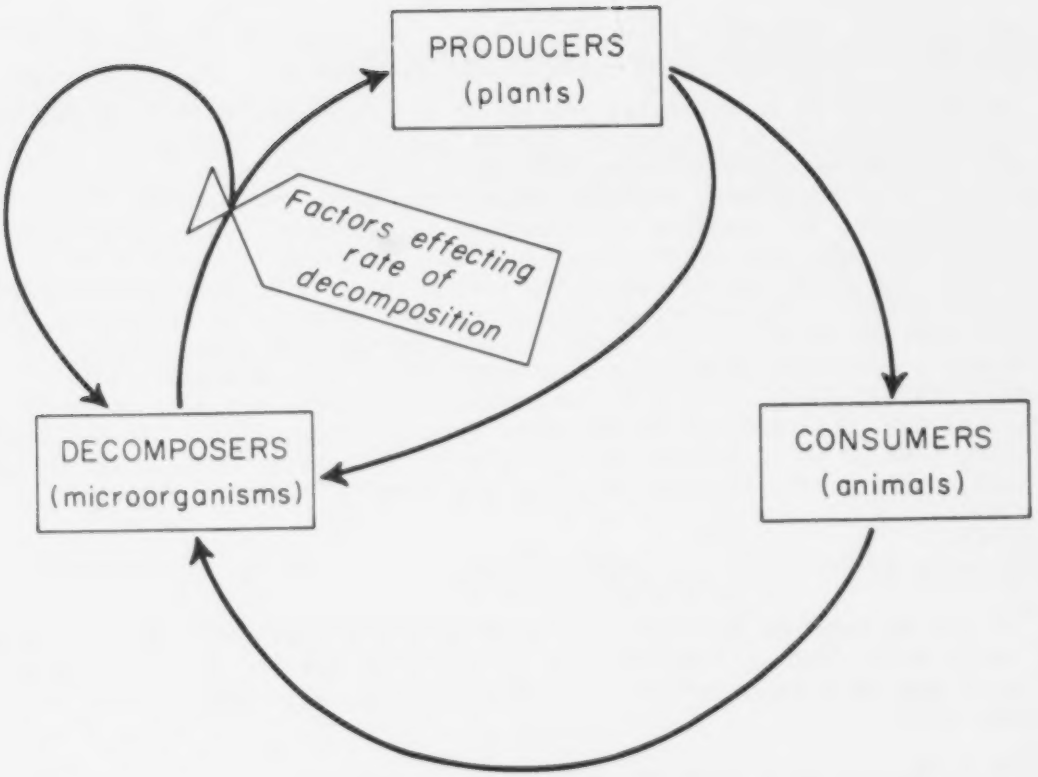

Figure 1. Simplified systems diagram of the organic matter cycle.

including nitrogen fixing bacteria (Wilson and Reisenauer, 1970); they affect the soil fungal flora (Hartman, 1974) and soil protozoa (Bojsova, 1963). Although little is known about heavy metal and nonmetal effects on the soil fauna, particularly the arthropods, high concentations of As and Cu in orchard and grassland soil have eliminated earthworms (van Rhee, 1973) while Cu^{++} and Ag^{+} were toxic to soil nematodes (Pitcher and McNamara, 1972; Hafkenscheild, 1971).

2. Community Evidence.

Pollutants may alter the natural rate of decomposition in soil systems with unknown, but crudely predictable, consequences. At low pollutant concentrations, a stimulation of the decomposition rate could result in more rapid remineralization. At higher concentrations, lethal effects on decomposer organisms result in cessation of decomposition and resultant plant growth in the affected area. Thus, plant growth rates and standing stock of animals might be expected to vary as a function of soil decomposition rates.

3. Field Evidence

Jackson and Watson (1976), and Watson et. al. (1976) found that litter accumulated and there were mineral nutrient abnormalities surrounding a lead smelter. They attribute these phenomena to disruption of the soil decomposition process. Dr. J. Wolak (Director, Forest Res. Inst., Warsaw, Poland, personal communication) has seen marked litter build-up in Polish forest soil near long standing smelters. Although the litter decomposer processes are largely inoperative, tree growth occurs to a limited extent. Apparently some essential nutrients for tree growth are in the air pollutants and/or these leach through the accumulated litter and carry soluable nutrient portions of the litter downward. Tyler has also shown a reduction in the decomposition in Swedish forests due to heavy metals emitted by local smelters (Tyler, 1972, 1974, 1975; Ruhling and Tyler, 1973).

C. Purpose.

The study of pollutant stressants on decomposition in extramural laboratories such as field plots suffers from several shortcomings that make the use of laboratory microcosms attractive. Variation over short distances in natural or prepared soils confounds treatment effects analysis, often negating definitive results (e.g., Bond, Russell, and Shimabuku, unpublished). To solve this problem, large and numerous field plots must be used, creating the problem of site decontamination after use of toxic and persistent pollutants. Even if an acceptable extramural site were found practical, this site would have only one of an almost infinite variety of soil types that would need to be studied.

Soil/litter microcosms can and have been designed small enough to allow many treatments with replication to be performed in a single experiment and the contaminated soil/litter is relatively easy to discard. Further, microcosms simulating a soil and/or litter system may be used to study nutrient or pollutant webs, pore sizes and transfer rates. Those data may be

useful in construction of predictive computer simulation models of the system. This document summarizes the soil/litter microcosm research conducted through March, 1977 at the Corvallis Environmental Research Laboratory.

II. METHODS

The microcosm system was designed and constructed (see Section II. A) with the following criteria in mind, and experimentally used to understand (see Section II. B.1), and evaluate pollutant effects (see Section II. B.2) on coniferous forest soil/litter systems. The criteria were: (a) simplicity of design and operation, (b) minimization of cost, (c) minimization of instrumentation size, (d) operation for extended time periods, (e) ability to measure biological "integrator-indicators,"1/ (f) ability to measure "differentiated-indicators,"2/ and (g) maximize studies of soil/litter microbiology.

Stotzky (1965), MacFadyen (1970), McGarity, Gilmore, and Bollen (1958), and Pramer (1965) discuss various aspects of soil microcosm design.

A. Description of Microcosm System Design.

The microcosm system consists of 10 "Life Supporting" modules (Figure 2). The "Life Support" system (Figure 3) for each microcosm consists of (a) an insulated microcosm or reactor chamber (Figure 4 and 33); (b) heat monitoring and maintenance module (Figure 5 and 34a); (c) carbon dioxide production monitoring module (Figure 5 and 34c); (d) internal pressure monitoring module (Figure 4 and 35); and (e) oxygen monitoring and generating module (Figures 6, 7, 36 and 37). 3/

The reactor chamber module consists of a one liter Dewar flask large enough to accept the glass microcosm insert (600 ml Berzelius glass beaker). The flasks were sealed with a foam stopper equipped lid with a small bore glass tube connected to the oxygen generator module, a thermal regulator 4/ and wires to a heating resistor connected to the mercury bulb of the thermal regulator. The thermal regulator and heating resistor were electrically inter-connected to maintain the temperature of the reactor chamber at the "set point" of the thermal regulator. Reactor heat loss and resistor heating constants were measured and used to evaluate heat production in each reactor.

1/ Integrator-indicators were defined as the community heat, oxygen, and carbon dioxide measurements.

2/ Differentiated-indicators were defined as the organism groups responsible for the changes in the integrator-indicators.

3/ The original prototype model of the device shown in Figure 7 was supplied by Dr. W. W. Anderson, Dept. of Microbiology, Oregon State Univ., Corvallis, OR.

4/ VWR, P.O. Box 3200, San Francisco, Calif., Catalog No. 61845-009.

(Dashed line components are for future development)

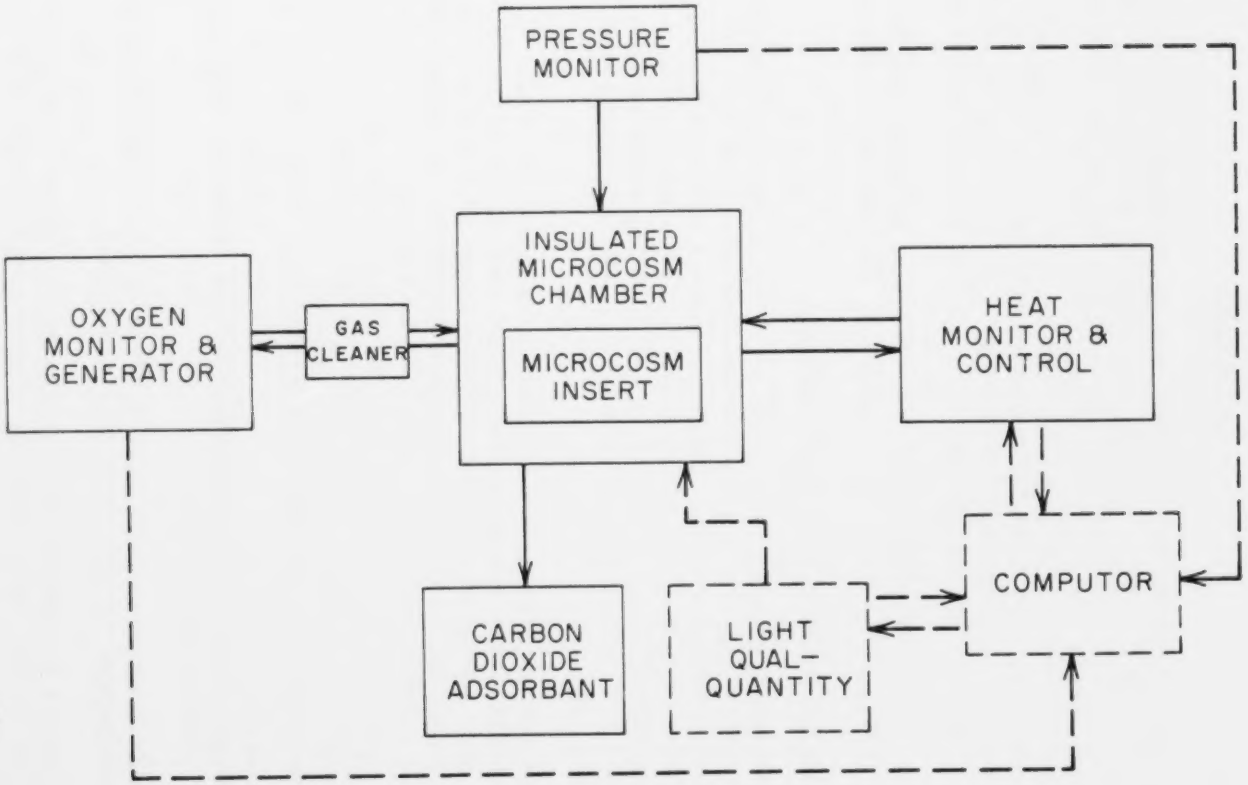

Figure 2. Systems diagram of the microcosm in its "life-support" system.

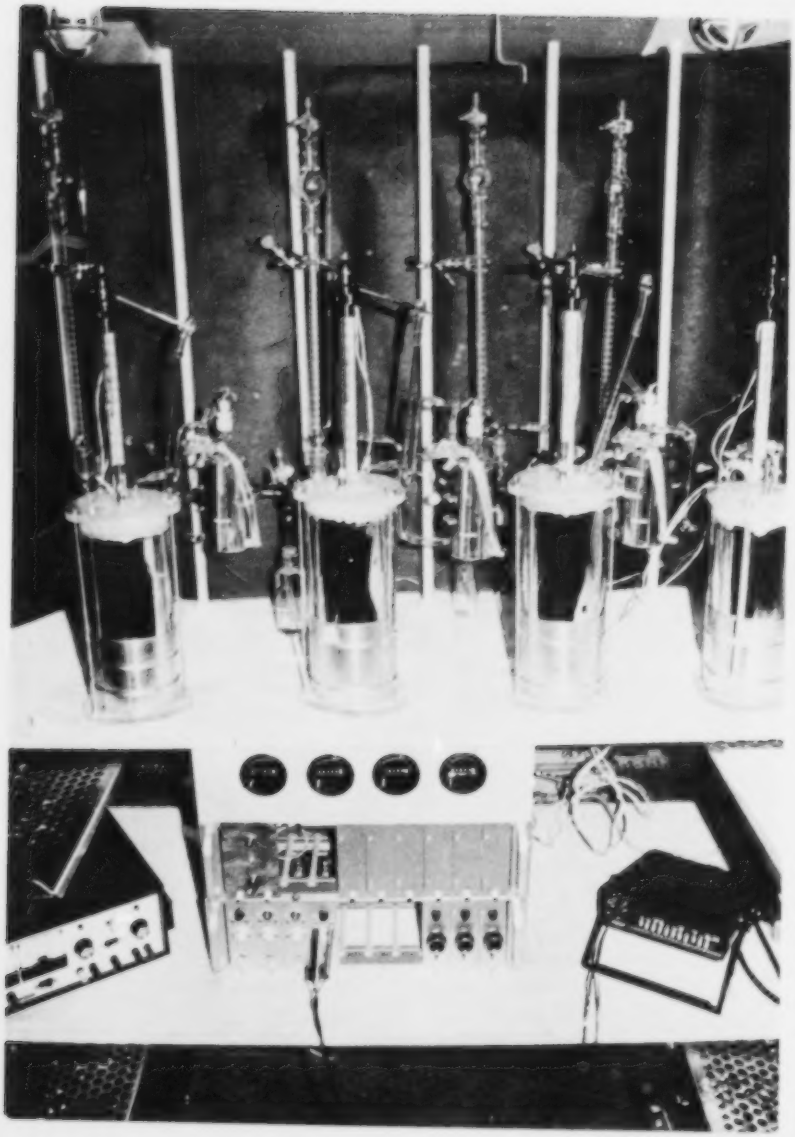

Figure 3. "Life support" systems.

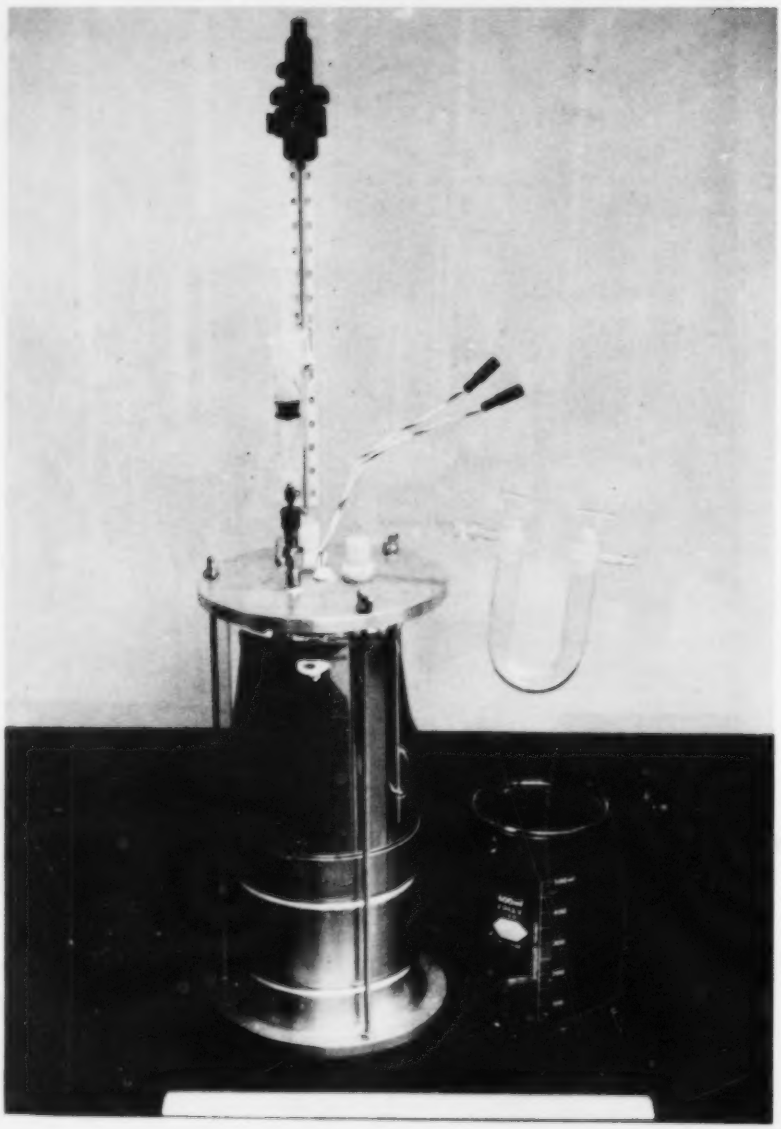

Figure 4. Single Reactor module.

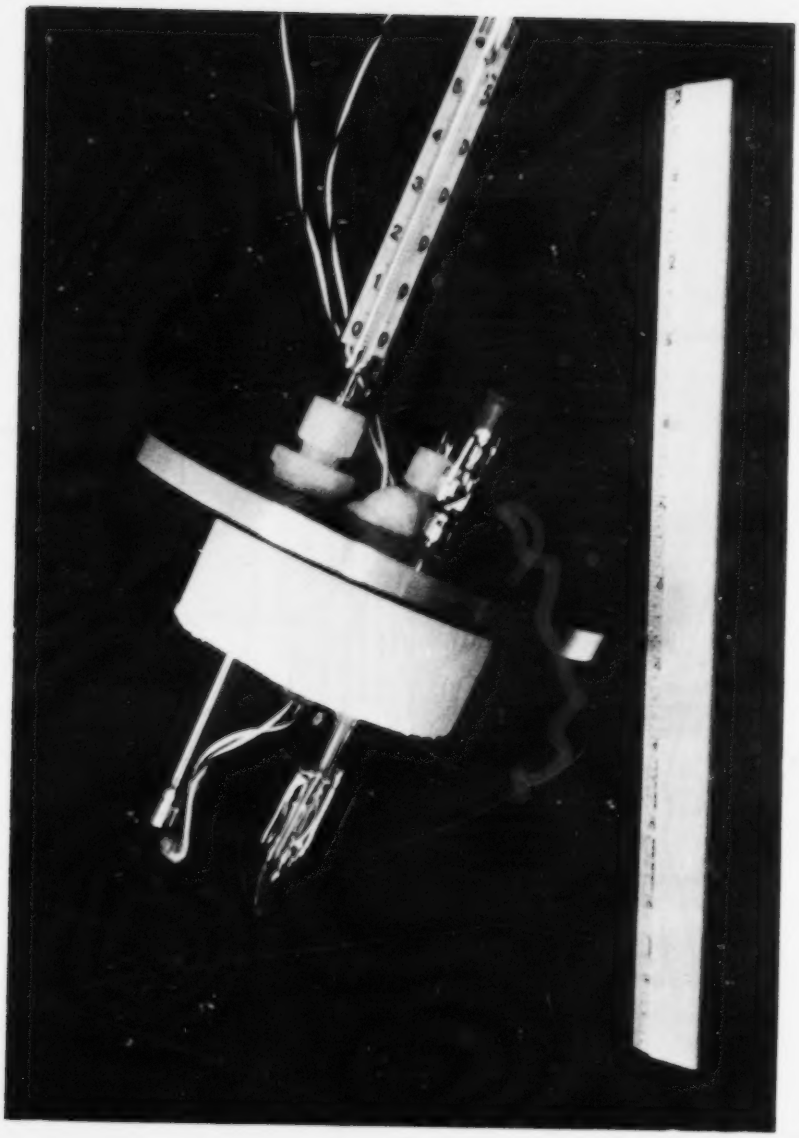

Figure 5. Close-up of reactor lid arrangement.

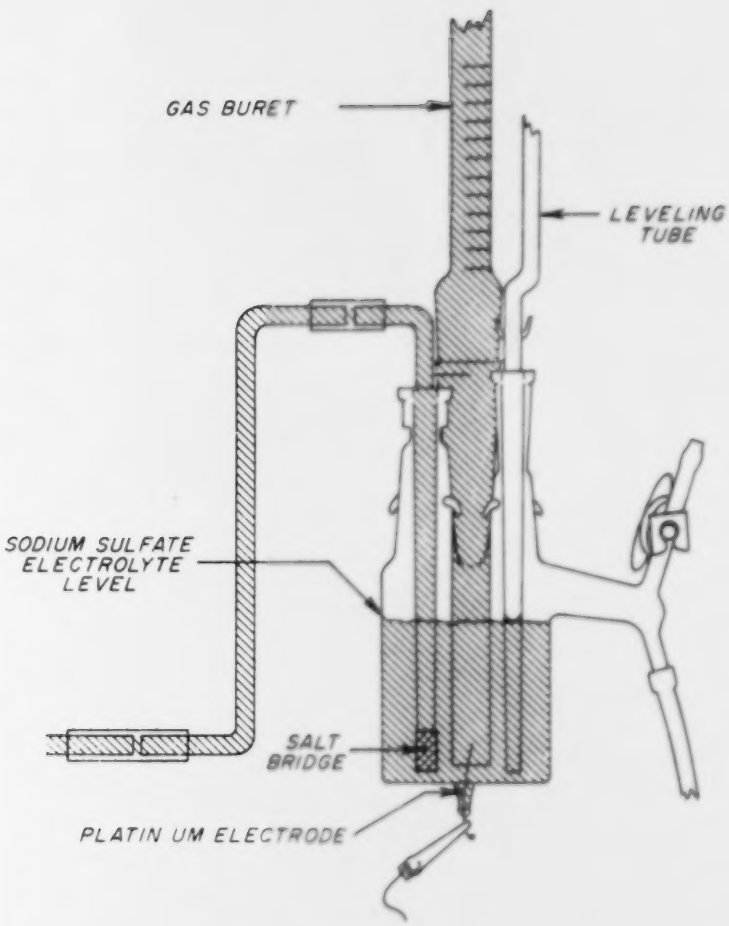

Figure 6. Diagram of hydrogen generator component of electrolytic oxygen generator showing salt bridge modification.

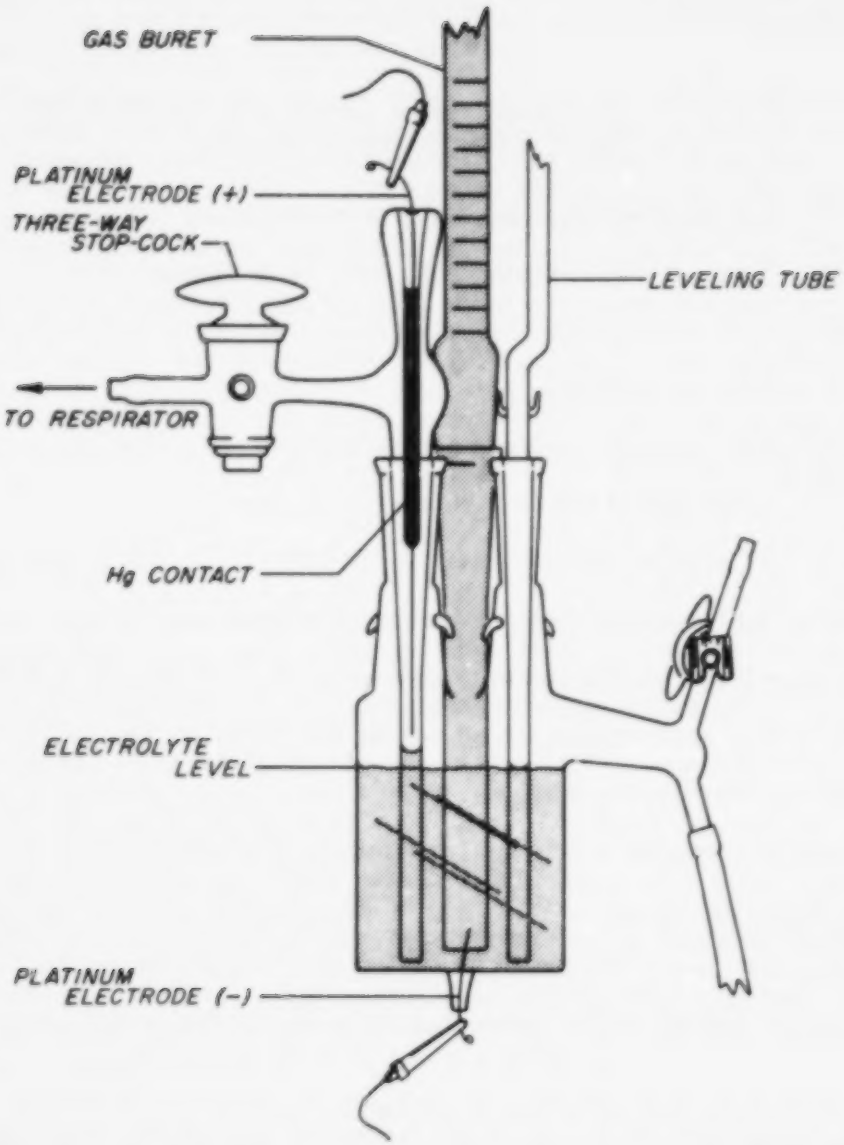

Figure 7. Diagram of electrolytic oxygen generator.

Biologically generated heat (in calories) was calculated and used in Equation 1:

$$\text{Biologically Generated Heat From Test Microcosm (in Calories)} = \frac{\Delta Q_t}{\Delta T} = \frac{K_t}{K_c} H_c \frac{\Delta t_c}{\Delta T} - H_t \frac{\Delta t_T}{\Delta T} \qquad \text{(Eq. 1)}$$

where: $\frac{K_t}{K_c} = \frac{H_t t_T}{H_c t_c}$ and is evaluated during a calibration run, i.e., using a non living microcosm,

$H = \frac{v^2}{R}$ Heating rate constant for resistor,

t = Time heating resistance is "on" in minutes,

T = Test reactor,

c = Control reactor,

Q = Heat output by microcosm in calories,

T = Experiment time in minutes,

v = Voltage at heating resistor in volts,

R = Resistance of heating resistor in ohms.

Carbon dioxide within the reactor was trapped in 10.0 ml of 0.60 N NaOH. Every 24 or 48 hours during an experiment the NaOH in the vial was withdrawn through a permanently placed needle and valve in the reactor vessel top and titrated (Coleman, 1973). Data are presented as milliliters carbon dioxide generated during the trapping period.

In an earlier version of the system, oxygen level maintenance and monitoring was performed with a hydrolytic oxygen generator similar to that designed by McGarity, et. al., 1958 (Figure 7). Oxygen consumed during microcosm respiration in the closed reactor system resulted in reduced internal pressure activating an electrolytic switch that causes oxygen to be delivered to the reactor. Hydrogen is stoichiometrically generated at the cathode. One-half of the hydrogen volume generated is equal to the oxygen produced at the anode.

The system described here uses a sodium sulfate/agar salt bridge embedded in fritted glass between the hydrogen and oxygen generating electrode, eliminating fluctuations in the electrolytic switch due to barometric pressure changes (Figure 6). A further modification in which oxygen will be generated at the anode and copper plated out at the cathode is now being tested. Ozone or other gases generated during the electrolytic reaction (Woodland, 1973) is trapped in activated charcoal and nickel dibutyldithiocarbamate impregnated glasswool located in a U-tube between the oxygen electrode and reactor chamber. Any gas leaks in the slightly positive pressure maintained system was monitored with a closed manometer system (Figure 4 and 35).

A detailed documentation of the "Life Support" system with scale drawings is presented in Appendix D.

B. Experimental.

The experiments described are not intended to be comprehensive but rather to illustrate principles of effects of natural and pollutant stress variables on soil/litter microcosm systems.

Initially four "Life Support" Systems were constructed and experiments were designed to observe (1) organismal populations succession between "identically" prepared and incubated microcosms, (2) variation in respiration, (3) detection of the initial flush phenomenon, (4) effects of microcosm soil moisture at 10, 30, and 60 percent of field water holding capacity on "indicator" microcosms. Another experiment (5) was designed to study the effects on soil respiration to treatment with three levels of cadmium (0.0, 0.01 and 10 mg $CdCl_2$/kg dry soil). Subsequently, eight microcosm systems were prepared to study the effects of (6) salt quality, i.e., the anions of chloride and sulfate of sodium, and the cations of lithium and sodium (chlorides), (7) the quantity at 0.132, 1.32, and 13.2 mM sodium chloride and 0.1761, 1.761 and 17.61 mM sodium sulfate, and three experiments to observe the respiratory effects of the following trace elements at high naturally found concentrations: (8) Cd, Cu, As, Se, Hg, Zn, Pb; (9) Ni, Cr, Co, Mn, Cd, Mo; and (10) Ca, V , Li, Sn, La, Sb, and Ag.

1. Natural Variables.

Microcosm vessels were prepared to contain 150 grams dry weight (DW) of homogenized and sifted soil, overlain with 15 grams (DW) of sifted litter. Replicate or "identical" microcosms were prepared by simultaneously sifting moisture-adjusted soil and litter components into a battery of microcosms until a predetermined weight of material was added. Soil and litter was compacted by setting a weighted cylinder on top of the material for one minute after each addition of soil or litter. Microcosms were then monitored in the soil ecosystem respirometer (SER) system to determine variability in respiration rates. Reactors were maintained at 20° C in a constant temperature room held at 17 to 18°C.

Surface spread plate counts of total heterotrophic bacteria and microfungi were used to determine succession potential by counting in triplicate on plates of 10-fold sterile phosphate buffer dilutions of agitated soil and litter samples on the following media: Acidified Potato Dextrose Agar (Difco, 1958) for fungi, and Bunt and Rovira's medium (Bunt and Rovira, 1955) for bacteria. Incubation was at 20°C for two weeks.

Analyses for soil and litter moisture were determined gravimetrically on 105°C oven-dried samples. In the soil moisture experiment, soil was moistened to the desired fraction of field water holding capacity prior to microcosm loading.

For the trace element and salt experiments, the test materials were introduced into the microcosm by injecting an aqueous solution of the substance into the soil and litter at 45 points with a syringe. See Table 2 for the chemical species and mean microcosm levels used. Also, see Appendix A for further preparation details.

2. Pollutant Stress

To determine if the biological age of microcosms was an influential factor on pollutant stress effects or on the establishment of controlled populations of Collembola or mites, identical microcosms were prepared and held at -4°C. After attaining the frozen state, groups were removed and incubated and/or monitored for respiratory activity at 0, 5, and 15 or 20 days of age, respectively; then all groups except the controls were spiked with $CdCl_2$ at 1500 ppm. Effects on populations planted at 0, 5, and 15 days were determined by intermittent and final destructive harvests.

Trace element quality, quantity and combination effects were assessed by spiking the prepared microcosms with aqueous solutions of the candidate pollutants at controlled concentrations either singly or in combination. Spiking (injection) techniques are described in Appendix A.

III. RESULTS AND DISCUSSION

A. Natural Variables.

In most instances the data will be presented and discussed in the integrated form, that is, as the slope of the summed measurements over time, e.g., the slope of the summation of oxygen consumed or carbon dioxide produced, over time in a microcosm. These slopes indicate production rates. When presented, populations of organisms will be referred to in differentiated terms or numbers of organisms.

Due to the inadaquacy of the biogenic heat measuring system at this writing it will be discussed only briefly.

1. Succession.

Population densities of microorganisms, particularly the bacteria in the microcosms, change dramatically immediately after microcosm preparation (Figure 8a). There is a rapid increase in the first 10 days -- from 10^7 to 10^8 bacteria/gm (DW) of soil or litter. The population remains almost constant at the higher level for at least 20 days longer.

This rapid microbial growth is also reflected in an initial rapid flush of CO_2 and uptake of O_2 in the first 10 days in the life support systems (Figures 9 and 10).

Jenkinson and Poulson (1976) found a similar initial flush and attributed it to disruption of previously inaccessible microbial nutrients released from destroyed organisms as a result of soil manipulation. In any event, it appears that relatively stable populations within the microcosms prevail 10 days after their preparation.

Therefore, all experiments were subsequently initiated only after a 10 day pre-incubation of the prepared microcosms. It was reasoned that the 10 day "flush" was an adjustment period and unless the interest was studying the effects of stressants on adjustment in the microcosms, the adjustment should be allowed to proceed to "completion" before stressing the systems. This would more closely test the stress effect which occurs in nature most of the time.

2. Microcosm Variation.

Specific experiments to test inter-microcosm variation and those portions of stress testing experiments with like treatments were used to evaluate the variation that occurs within and among "identically" prepared and incubated microcosms (e.g., Figures 11 and 12).

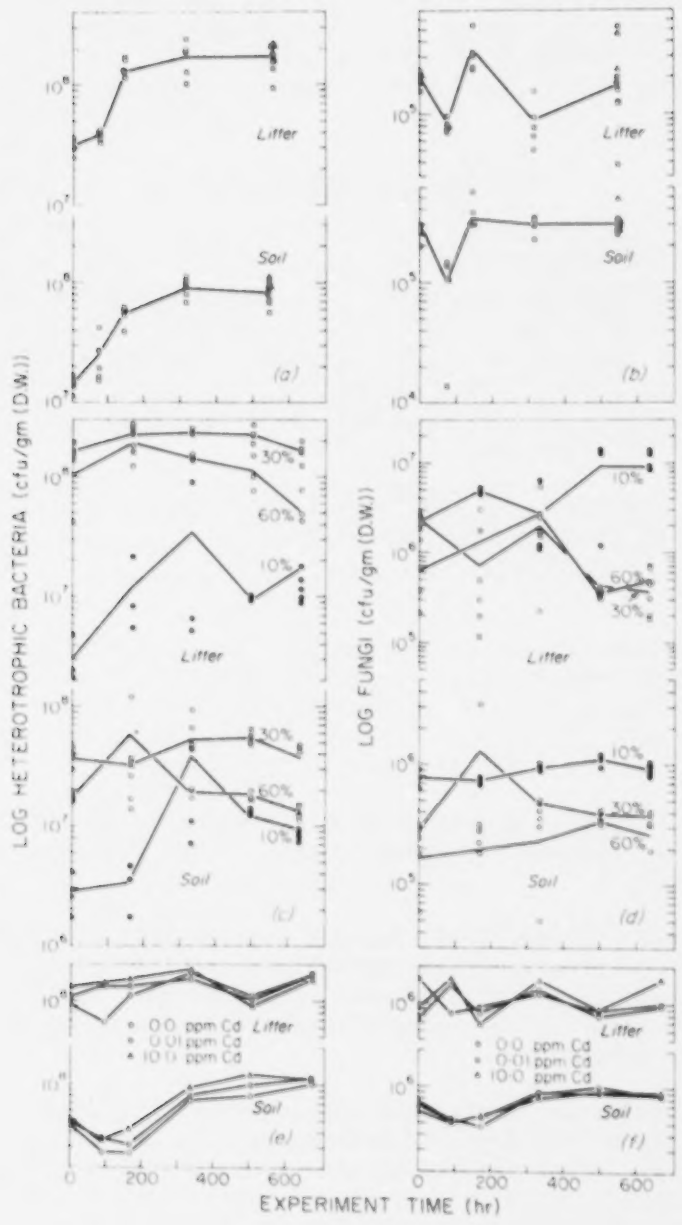

Figure 8. Graphs of heterotrophic bacterial and fungal propogules (CFU = colony forming units) in soil and litter in three "identically" processed microcosms (a & b); three "identically" processed microcosms incubated at 10, 30 and 60% of field water holding capacity, respectively (c & d); and three "identically" processed microcosms injected with distilled water (0.0), 0.01 and 10.0 ppm final mean $CdCl_2$ concentration, respectively (e & f).

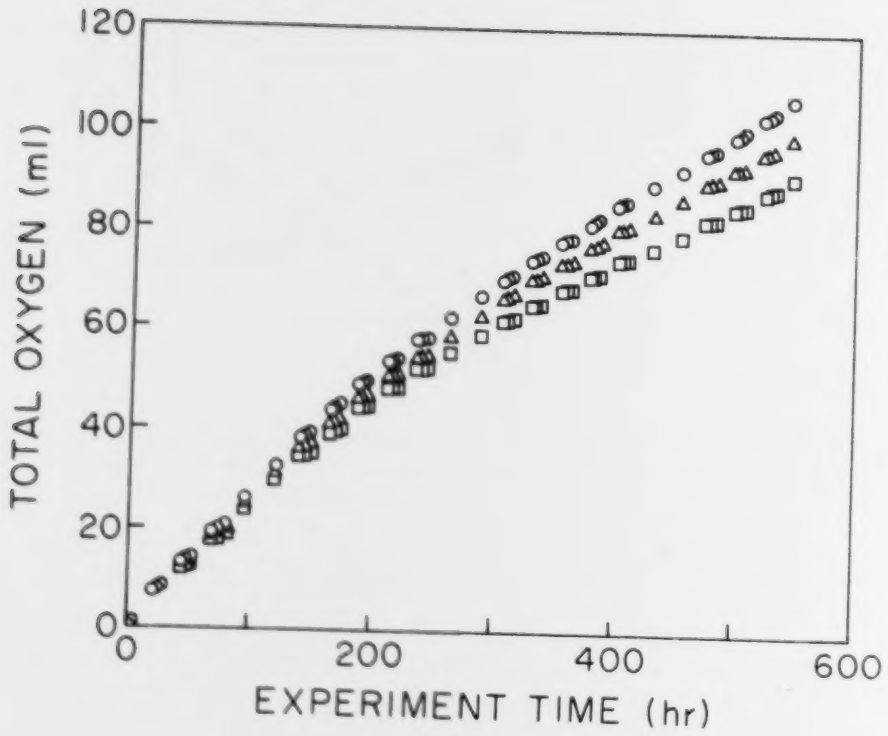

Figure 9. Accumulated oxygen consumed through time in three "identically" prepared and incubated coniferous forest soil/litter microcosms.

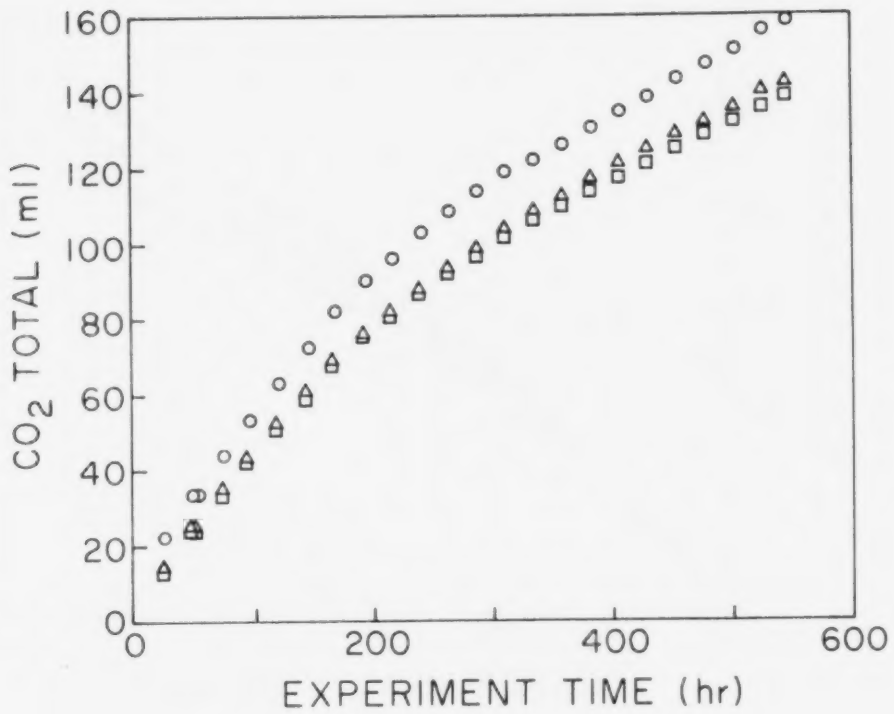

Figure 10. Accumulated carbon dioxide produced through time in three "identically" prepared and incubated coniferous forest soil/litter microcosms.

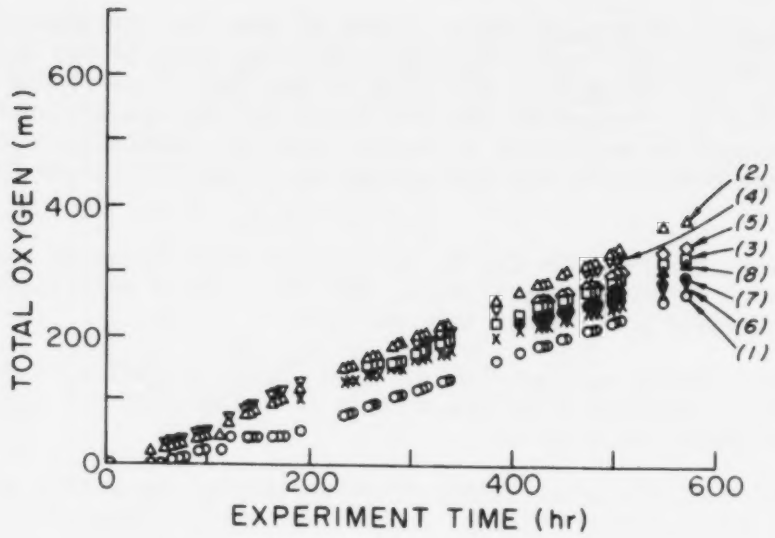

Figure 11. Accumulated oxygen consumed through time in the 8 indicated and "identically" prepared and incubated coniferous forest soil/litter microcosms.

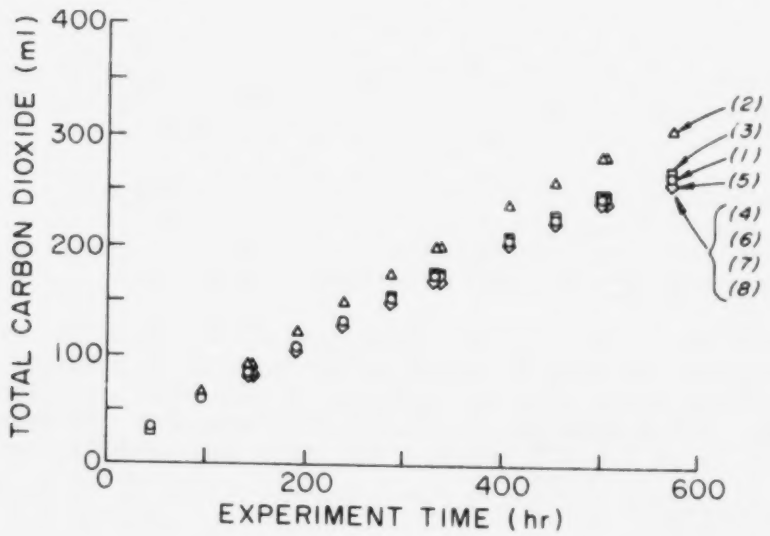

Figure 12. Accumulated carbon dioxide produced through time in the 8 indicated, and "identically" prepared and incubated coniferous forest soil/litter microcosms.

The results of 29 experiments (Table 1) show that the mean rates of oxygen consumption and carbon dioxide production are 0.54 and 0.43 ml/hr, respectively, for 150 gm (DW) soil plus 15 gms (DW) litter. The variances of 0.05 ml/hr for O_2 consumption and 0.03 ml/hr for CO_2 production indicate that seasonal changes of metabolism in natural soil are undetectable in these systems after preparation and pre-incubation in the microcosms for 10 to 14 days.

The large variance for the O_2 consumption rate is due to the relatively less sensitive method of measuring O_2 than CO_2. It is anticipated that system improvement will reduce this variance.

The respiratory quotient (RQ) of 0.80 (Table 1) indicates the mean metabolism of compounds is at the protein to lipid oxidation state (White, Handler and Smith, 1973, p. 283).

The coefficients of variation (CV) for the O_2 consumption and CO_2 production rates provides a means to estimate a significant difference between the control rates and the test rates in any single set of microcosms, i.e., the CV's from these data can be used to determine what may be called a 95 percent confidence level about a test set control rate (Eq. 4).

$$P\{ (-2*CV_i) * \text{microcosm} \leq \text{Microcosm} \leq (2*CV_i) * \text{microcosm}\} = 0.95 \quad \text{(Eq. 4)}$$

where i is the CV as calculated from the data found in Table 1.

3. Soil Moisture.

The effect of soil moisture on microbial populations is shown in Figure 8c and 8d where the tendency is for the bacterial populations to prevail under moist conditions and fungi under drier conditions.

The integrated biological activity as deduced from oxygen consumption and carbon dioxide production (Table 2, Figures 13 and 14) shows a marked increase with increased soil moisture, i.e., respiration was two to three times faster at 60 percent than 10 percent soil moisture.

From the RQ's (Table 2) it is seen that at 30 and 60 percent soil moisture, respiration indicates a "usual" oxidation process compared to other results in Table 2, but that at 10 percent a more oxidizable substrate may be metabolized. These observations may be correlated with the relatively high fungal activity at low soil moisture and their saccharophilic reputation.

4. Salt Effects.

As seen in Figures 15, 16, 17 and 18 and tabulated in Table 2, the quality and quantities of salts, or rather anions, may markedly inhibit community respiration in the coniferous forest soil/litter microcosms. The respiratory relationship of CO_2 production and O_2 consumption is not affected by salt treatment as indicated by the small differences in RQ's between microcosm treatments. However, there is a substantial anion effect that is

TABLE 1. MEAN (BAR); STANDARD DEVIATIONS (S) AND COEFFICIENTS OF VARIATION (CV) FOR OXYGEN CONSUMPTION (X_1), CARBON DIOXIDE PRODUCTION (X_2), AND RESPIRATORY QUOTIENT (X_1/X_2) FOR ALL OF NON TREATMENT PORTIONS WITHIN AND AMONG "IDENTICALLY PREPARED AND INCUBATED SETS OF CONIFEROUS FOREST SOIL/ LITTER MICROCOSMS

Experiment Number	Number in Set	$\bar{X}_1$	S_{X_1}	CV_{X_1} (%)	$\bar{X}_2$	S_{X_2}	CV_{X_2} (%)	$(\bar{X}_1/\bar{X}_2)$	$S_{\bar{X}_1/\bar{X}_2}$	$CV_{\bar{X}_1/\bar{X}_2}$
13	7	0.55 1/	0.07	12.9	0.41 1/	0.01	1.7	0.77	0.09	11.9
14	8	0.48	0.06	12.0	0.40	0.01	4.0	0.83	0.11	12.8
18	2	0.46	0.00	0.9	0.39	0.01	3.4	0.84	0.02	2.5
18	4	0.55	0.08	14.8	0.45	0.03	6.5	0.82	0.08	10.2
22	8	0.59	0.05	7.8	0.47	0.02	4.5	0.79	0.09	4.9
Total Number		29		29	29		29	29		29
Mean of Means		0.54		10.7	0.43		3.9	0.80		8.6
S of Means		0.05		3.7	0.03		1.5	0.03		4.4

1/ ml/hr

TABLE 2. TABULATION OF RESPIRATORY RATES FOR "IDENTICALLY" PREPARED AND INCUBATED CONIFEROUS FOREST SOIL/LITTER MICROCOSMS WITHIN EACH EXPERIMENT, AND SUBJECTED TO THE INDICATED TREATMENTS.

Microcosm Treatment	Mean Microcosm Concentration (ppm[1])	Oxygen Consumption Rates (ml/hr)		Carbon Dioxide Production Rate (ml/hr)		Respiratory Quotient (CO_2/O_2)	
		Pre-[2]	Post-[2]	Pre-	Post-	Pre-	Post-
Experiemnt 14							
Control	0	0.508	0.449	0.385	0.361	0.76	0.80
$CdCl_2$	1500	0.499	0.196***	0.397	0.200***	0.80	1.02
$CoCl_2$	3000	0.537	0.416	0.402	0.184***	0.75	0.44
Na_2HAsO_4	1000	0.550	0.526	0.403	0.364	0.73	0.69
SeO_2	680	0.440	0.250***	0.368	0.213***	0.84	0.85
$HgCl_2$	30	0.376	0.345	0.391	0.276***	1.04	0.80
$ZnCl_2$	7500	0.432	0.148***	0.404	0.109***	0.94	0.74
$PbCl_2$	200	0.518	0.414	0.413	0.348	0.78	0.84
Experiment 16							
Control	0	-----	0.484	-----	0.356	----	0.74
$NiCl_2$	700	-----	0.250***	-----	0.174***	----	0.70
$CrCl_2$	100	-----	0.385	-----	0.255***	----	0.66
$CuCl_2$	50	-----	0.380	-----	0.292***	----	0.77
$MnCl_2$	850	-----	0.252***	-----	0.206***	----	0.82
$CdCl_2$	1500	-----	0.217***	-----	0.165***	----	0.76
MoO_3	10	-----	0.540	-----	0.414	----	0.77
Experiment 22							
Control	0	0.585	0.510	0.498	0.397	0.77	0.78
$CaCl_2$	6131	0.591	0.235***	0.472	0.164***	0.80	0.70
V_2O_5	60	0.611	0.481	0.469	0.379***	0.77	0.79
LiCl	100	0.581	0.331**	0.472	0.264***	0.81	0.80
$SnCl_2$	10	0.573	0.503	0.438	0.385	0.77	0.77
$LaCl_3$	100	0.545	0.425	0.468	0.355***	0.86	0.83

TABLE 2 (CON'T)

Microcosm Treatment	Mean Microcosm Concentration (ppm[1])	Oxygen Consumption Rates (ml/hr) Pre-[2]	Oxygen Consumption Rates (ml/hr) Post-[2]	Carbon Dioxide Production Rate (ml/hr) Pre-	Carbon Dioxide Production Rate (ml/hr) Post-	Respiratory Quotient (CO_2/O_2) Pre-	Respiratory Quotient (CO_2/O_2) Post-
$SbCl_3$	10	0.532	0.457	0.459	0.400	0.86	0.88
$AgNO_3$	10	0.683	0.606	0.506	0.442***	0.74	0.73
Experiment 13[3]							
Control 1	---	0.568	-----	0.407	-----	0.717	----
Control 3	---	0.559	-----	0.417	-----	0.746	----
Control 4	---	0.662	-----	0.418	-----	0.633	----
Control 5	---	0.517	-----	0.404	-----	0.781	----
Control 6	---	0.447	-----	0.402	-----	0.900	----
Control 7	---	0.473	-----	0.416	-----	0.879	----
Control 8	---	0.537	-----	0.407	-----	0.758	----
Experiment 3							
Moisture	10%	0.014	-----	0.028	-----	1.10	----
Moisture	30%	0.050	-----	0.056	-----	0.76	----
Moisture	60%	0.122	-----	0.091	-----	0.75	----
Experiment 18a[3] Long term variation (900 hrs)							
Microcosm 5	---	0.455	-----	0.377	-----	0.83	----
Microcosm 6	---	0.460	-----	0.395	-----	0.86	----
Experiment 18b Short term variation							
Microcosm 5	---	0.508	-----	0.438	-----	0.86	----
Microcosm 6	---	0.459	-----	0.421	-----	0.92	----
Microcosm 7	---	0.616	-----	0.448	-----	0.73	----
Microcosm 8	---	0.627	-----	0.489	-----	0.78	----

TABLE 2 (CON'T)

Microcosm Treatment	Mean Microcosm Concentration (ppm[1])	Oxygen Consumption Rates (ml/hr) Pre-[2]	Post-[2]	Carbon Dioxide Production Rate (ml/hr) Pre-	Post-	Respiratory Quotient (CO_2/O_2) Pre-	Post-
Experiment 24							
Na_2SO_4	0.1761 mM	0.430	0.417	0.408	0.338	0.95	0.81
Na_2SO_4	1.761 mM	0.459	0.361	0.414	0.282	0.90	0.78
Na_2SO_4	17.61 mM	0.534	0.308	0.446	0.235	0.84	0.76
NaCl	0.132 mM	0.516	0.444	0.401	0.347	0.78	0.78
NaCl	1.32 mM	0.404	0.229	0.386	0.199	0.96	0.87
NaCl	13.2 mM	0.430	0.185	0.382	0.145	0.89	0.78
LiCl	552.0 ppm	0.481	0.319	0.470	0.293	0.98	0.92
H_2O	---	0.540	0.473	0.422	0.357	0.78	0.76
Mean		0.474	0.342	0.416	0.275		
Variance		0.052	0.102	0.030	0.076		
Coe. of Variation (%)		11.0	29.8	7.2	27.7		

1 As metal or non metal

2 Pre- and Post-pollutant treatment

3 Rate calculated from entire experimental time.

** $\geq$ 2 Coefficients of variation from experiment control.

*** $\geq$ 3 Coefficients of variation from experiment control.

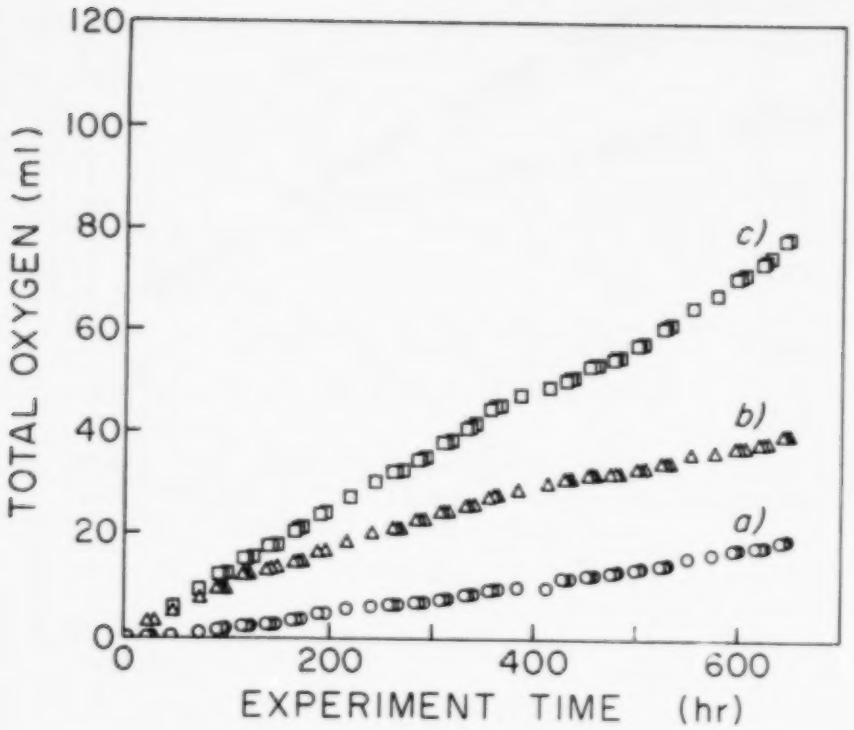

Figure 13. Accumulated oxygen consumed through time in three "identically" prepared and incubated coniferous forest soil/litter microcosms treated to an initial soil moisture of (a) 10%, (b) 30%, (c) 60% of field holding capacity.

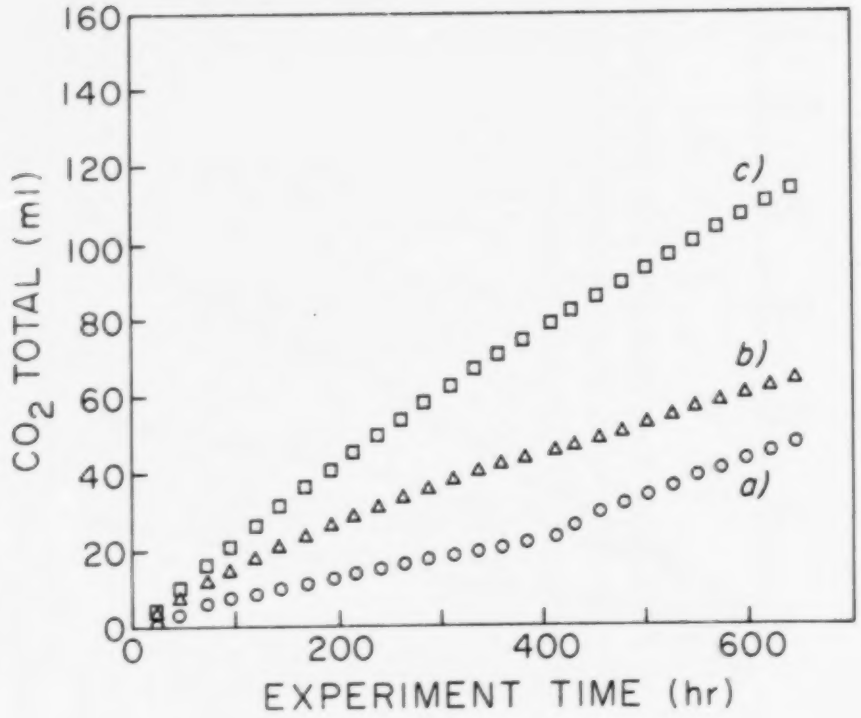

Figure 14. Accumulated carbon dioxide produced through time in three "identically" prepared and incubated coniferous forest soil/litter microcosms treated to an initial soil moisture of (a) 10%, (b) 30%, (c) 60% of field holding capacity.

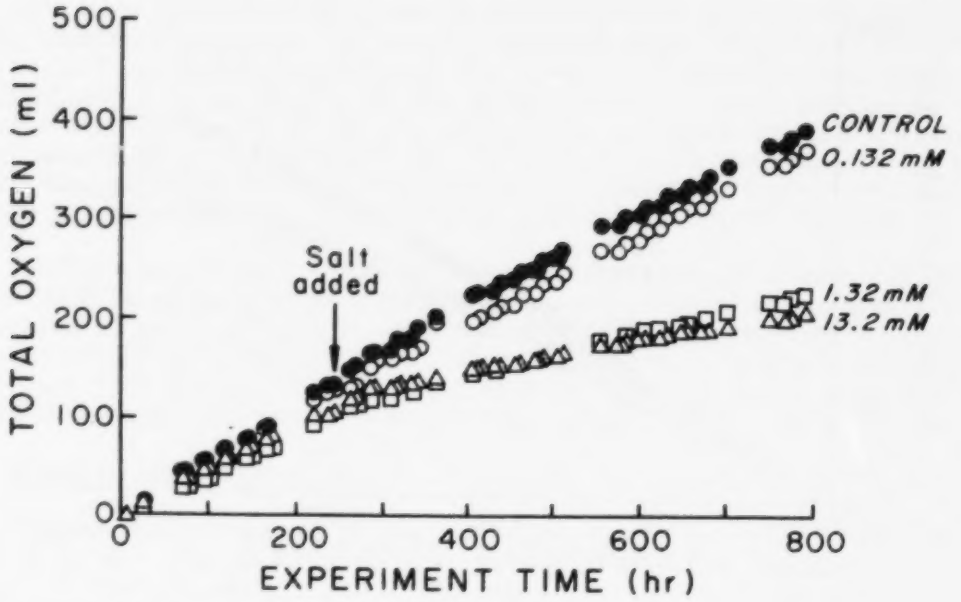

Figure 15. Accumulated oxygen consumed through time in "identically" processed coniferous forest microcosms treated to a final mean indicated concentration of sodium chloride.

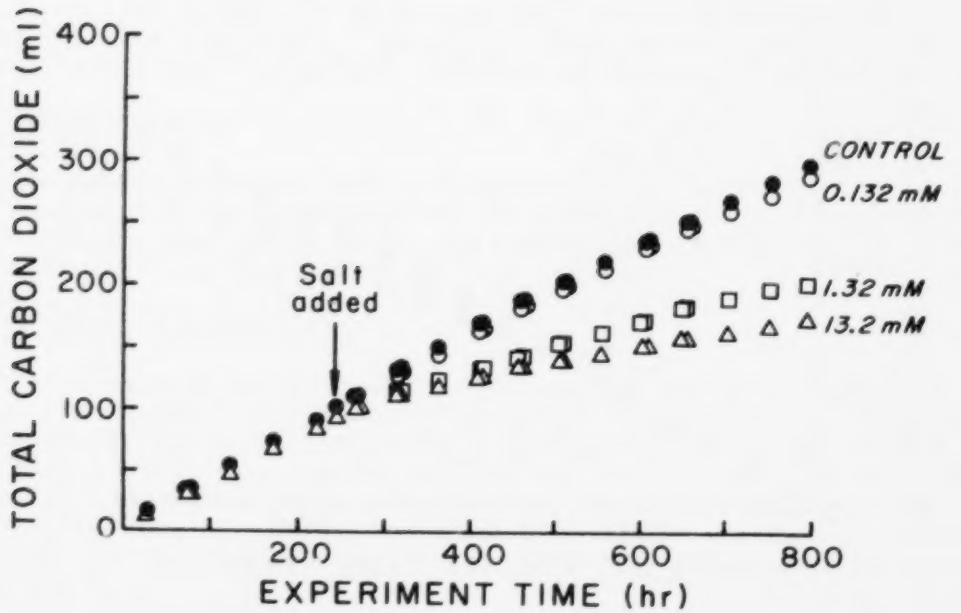

Figure 16. Accumulated carbon dioxide produced through time in "identically" processed coniferous forest microcosms treated to a final mean indicated concentration of sodium chloride.

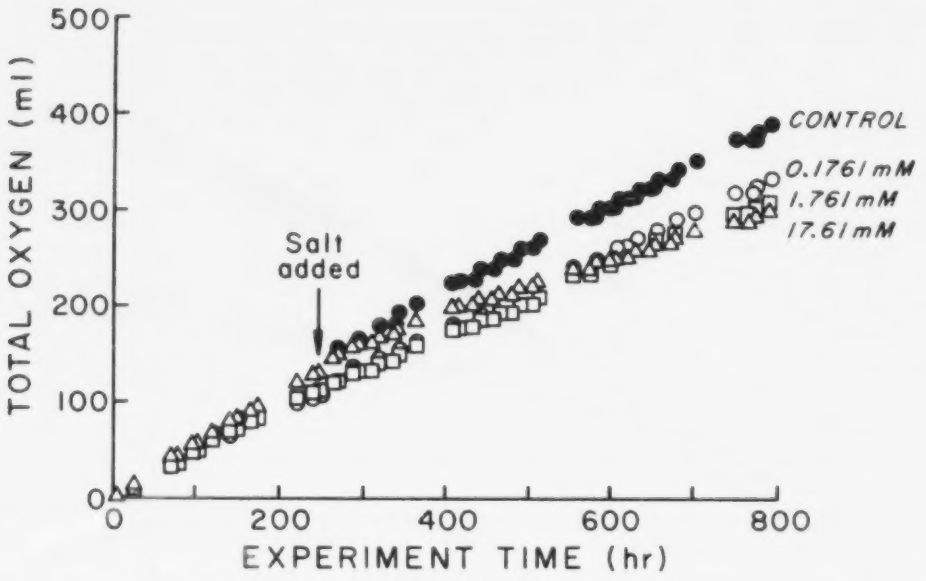

Figure 17. Accumulated oxygen produced through time in "identically" processed coniferous forest microcosms treated to a final mean indicated concentration of sodium sulfate.

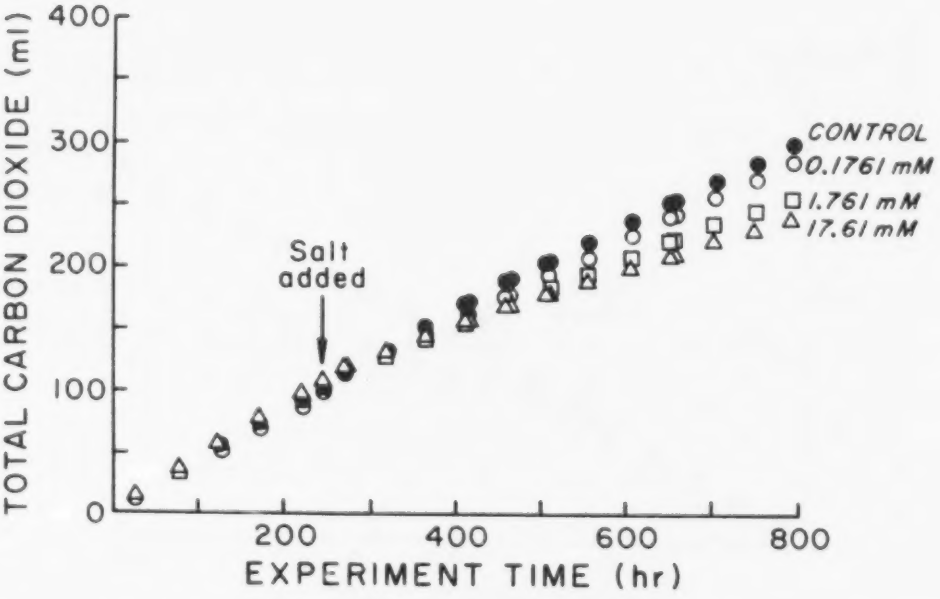

Figure 18. Accumulated carbon dioxide produced through time in "identically" processed coniferous forest microcosms treated to a final mean indicated concentration of sodium sulfate.

seen as a more pronounced decrease in respiration in the NaCl as opposed to Na_2SO_4 treated microcosms. On a molar basis it might be a logarithmic function of salt concentration (Figure 19). From this experiment one could conclude that a large portion of the effects of toxic trace substances added to soils may not be due to the toxicity of the trace substance but rather an effect of a high salt concentration. Of course, a combination of high salt and presence of specific toxic substances may synergize their individual effects resulting in more than additive respiratory inhibition.

Further comparison of Na_2SO_4 treatment salt effects (Figure 19) with a 6.7 mM $MgSO_4$ treatment gives a 25 and 33 percent reduction in respiration, respectively, whereas there is an 8 percent difference based on molarity. In further calculations based on ionic strength, there is only a 3 percent difference. It appears that at least at high toxicant levels a salt effect may be the dominating process causing respiratory loss in the soil community.

5. Biogenic Heat Production.

Biogenic heat produced by the microcosm community in the "salts effects" experiments was detected (Table 3) where positive values are shown for all changes in heat production rates. However, the trends in the changes in the heat and CO_2 production rates do not correlate for both NaCl and Na_2SO_4 series as one might expect. Further, the coefficient of variations for the pre- and post-treatment of biogenic heating rate are the same, indicating only random variation causing rate changes. It is reasoned that the amount of biogenic heat produced by the microcosms is small compared to the total heat input to the system as seen from equation (1). The calculation of biogenic heat is markedly subject to small errors in measurement of heating resistor on-time heat input. Thus, it is shown that the quantification of biogenic heat is still uncertain. Efforts are underway to solve this problem.

B. Pollutant Stress.

The desired microcosm biological age at which to add pollutant stresses (Section III. B.1) was determined and the effects of the quality (Section III. B.2), quantity (Section III. B.3), and combinations (Section III. B.4) of pollutants are illustrated.

1. Microcosm Biological Age.

When microcosms are treated with 1500 ppm Cd^{++} at 0, 5 and 20 days incubation after preparation O_2 consumption and CO_2 production is reduced 22 - 30-30% and 38 -43-35%, respectively (Table 4, Figure 20). Based on the RQ data which remains relatively constant at 0.71 - 0.75 there is no obvious relationship between biological age and respiration. Because no regular pattern in the microbial population stress effect with microcosm age has been detected, we assume that the time of stress on microcosm respiratory activity is relatively small.

For 25 adult Collembola (Isotoma sp.), and approximately 3000 free living nematodes added to these same microcosms, the time after pollutant addition was critical. That is, pollutant added similtaneously with the

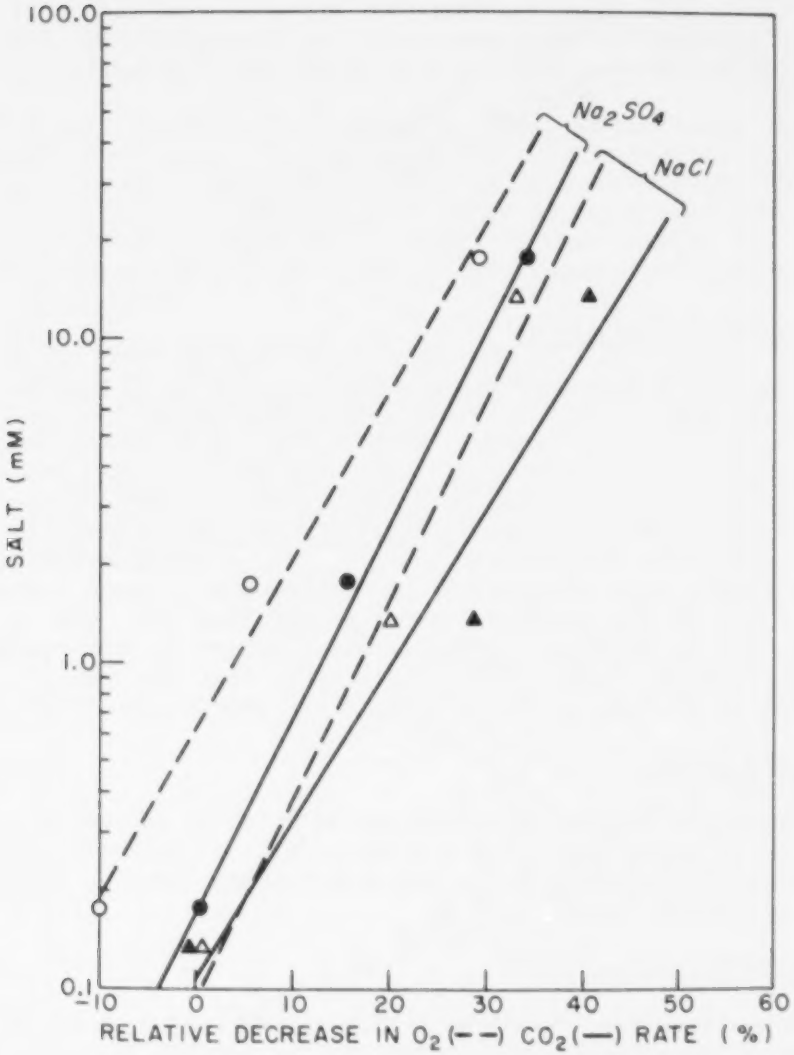

Figure 19. Salts (NaCl, Na_2SO_4) effects (compared to initial control rate) on respiration in "identically" prepared coniferous soil/litter microcosms treated to the indicated final mean salt concentration.

TABLE 3. TABLE SHOWING COMPARISON OF CARBON DIOXIDE PRODUCTION RATE AND BIOGENIC HEATING RATE (AS INDICATED BY MICROCOSM HEATER ON-TIME RATE) CHANGES DUE TO MICROCOSM TREATMENT WITH VARIOUS SALT CONCENTRATIONS.

Treatment	Biogenic Heat: Microcosm On-Time Heater Rate (hr/hr) Pre-Treatment	Biogenic Heat: Microcosm On-Time Heater Rate (hr/hr) Post-Treatment	Biogenic Heat: % Change	CO_2 Production: Microcosm CO_2 Rate Production (hr^2/hr) Pre-Treatment	CO_2 Production: Microcosm CO_2 Rate Production (hr^2/hr) Post-Treatment	CO_2 Production: % Change
No Microcosm	0.700	0.700	0	0	0	0
H_2O	0.287	0.296	3	0.422	0.355	15.8
0.1761 mM Na_2SO_4	0.414	0.450	9	0.408	0.338	17.0
1.761 mM Na_2SO_4	0.414	0.477	15	0.414	0.282	32.0
17.61 mM Na_2SO_4	0.315	0.394	25	0.446	0.235	47.3
0.132 mM NaCl	0.337	0.427	27	0.401	0.347	12.9
1.32 mM NaCl	0.404	0.477	18	0.386	0.199	48.5
13.2 mM NaCl	0.466	0.513	14	0.382	0.145	62.0
552.0 ppm LiCl	0.369	0.404	9	0.470	0.293	37.6
Mean	0.375	0.432		0.416	0.274	
Variance	0.060	0.071		0.030	0.076	
Coe. of Variation (%)	16	16		7	28	

TABLE 4. RESPIRATORY MEASUREMENTS OF "IDENTICALLY" PREPARED CONIFEROUS FOREST SOIL/LITTER MICROCOSMS TREATED AT THE INDICATED BIOLOGICAL AGES TO 1500 PPM $CdCL_2$.

Microcosm Biological Age at Treatment (Days)	Oxygen Consumption Rate (ml/hr)		Difference From Control (%)	Carbon Dioxide Production Rate (ml/hr)		Difference From Control (%)	Respiratory Quotient (CO_2/O_2)	
	Control	Test		Control	Test		Control	Test
0	0.55	0.43	-22	0.45	0.28	-38	0.81	0.75
5	0.46	0.32	-30	0.40	0.23	-43	0.86	0.71
20	0.43	0.30	-30	0.34	0.22	-35	0.80	0.74

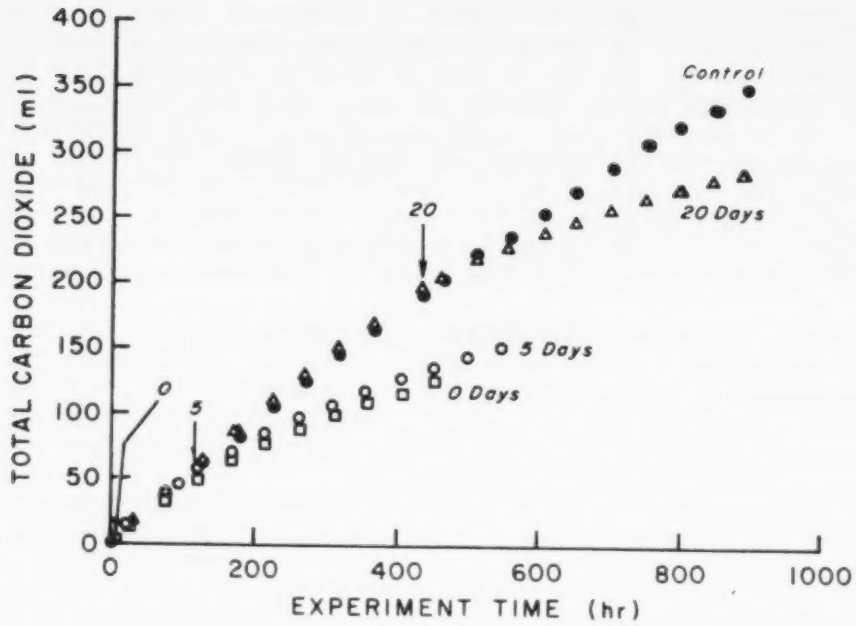

Figure 20. Accumulated carbon dioxide produced through time from "identically" prepared and incubated coniferous forest soil/litter microcosms treated to 1500 ppm $CdCl_2$ at either 0, 5 or 20 days of biological age.

organisms (0 day experiment) or within 1 week (5 day experiment) showed little or no growth of Collembola and a 98 percent decrease in nematodes after 20-25 days incubation (Table 5, Figures 21 and 22). Later pollutant additions resulted in the same marked population reduction effects. It appears that this pollutant stress has the same general characteristics for both animals regardless of the biological age of the system: cessation of reproduction and death of adults.

2. Trace Element Quality.

Statistically significant (Table 2) effects of particular trace elements added as single salts to the microcosm system show only an inhibition of CO_2 production by Cu, Hg, Cr, Co, V, and La while As showed a stimulation, whereas both CO_2 production and O_2 consumption were inhibited by Cd, Se, Zn, Ni, Mn, and Li. It is possible that oxygen consumption is also inhibited for the former group but the analytic methods cannot detect the change. It does appear that the statistical method used to limit significance in this case causes this result because the RQ's appear to be logical, i.e., about 0.7 to 0.8. Additionally, none of the RQ's except Experiment 1 Cd and Co are outside two coefficients of variation (see Table 1) from experiment controls.

Finally, graphical interpretation of Figures 23, 24, 25, and 26 show possible additional inhibitory effects of Cr, Hg, and Co on oxygen consumption and stimulation of oxygen consumption and carbon dioxide production by As and Mo. Copper causes an initial stimulation after pollutant addition and subsequently an inhibition of oxygen consumption and CO_2 production.

In conclusion it appears that trace elements found in real world levels can and probably do inhibit soil decomposition processes as seen by reduced respiratory activity.

3. Trace Element Quantity.

Respiratory response (Table 2, Figures 27 and 28) but not detectable microbial response (Figure 8e, f) varied with amount of cadmium added to microcosms and with time of observation. At low cadmium concentrations (0.01 ppm $CdCl_2$), a transient stimulatory effect (increased respiratory rate) was noted for the initial 200 hours after amendment, followed by recovery to control rates. Respiration at high cadmium concentrations (10 ppm $CdCl_2$) equaled that of the control for approximately 300 hours after amendment; thereafter, respiration decreased by 40 percent compared to the control (Bond, et al, 1975, 1976). The mechanism of the dramatic loss of activity at high cadmium levels is unknown. There was no decrease in numbers of culturable soil or litter fungi or bacteria, although there was a relative increase in oxygen consumed over carbon dioxide produced (Figure 29) suggesting uncoupling of oxidative phosphorylation; a common heavy metal effect (White, et al., 1973). Stimulation at low levels and inhibition at higher levels by heavy metals is a common microbial response to heavy metals, and is referred to as oligodynamic action (Salle, 1973) or the Arndt-Schulz Law (Lamanna and Mallette, 1965, p. 897).

TABLE 5. TABULATION OF THE SURVIVAL OF NEMATODES (CEPHALOBUS PERSEGNIS) AND COLLEMBOLA (ISOTOMA SP.) ADDED TO CONIFEROUS FOREST SOIL/LITTER MICROCOSMS AT THE INDICATED AGES, AND TREATED TO 1500 PPM $CdCl_2$ AT THE INDICATED MICROCOSM AGES.

Kind of Organisms Added to Microcosms		Number Added	Microcosm Age at Stress (Days)		% Viable After Indicated Microcosm Age (Days)					
Arthropods	Nematodes		Animals	$CdCl_2$	0	5	15	20	25	35
+	-	25	5	-5	100	60	28	--	--	--
+	-	25	0	0	100	60		12	--	--
+	-	25	0	5	100	96	332	--	116	--
+	-	25	0	15	100	66	132	--	1100	400
+	-	25	0	NA	100	66	132	--	1780	2140
-	+	3400	5	-5	100	19	0.9	--	--	--
-	+	3500	0	0	100	34		0.5	--	--
-	+	3000	0	5	100	31	18	--	3	--
-	+	3000	0	15	100	37	60	--	12	16
-	+	3000	0	NA	100	37	60	--	43	44

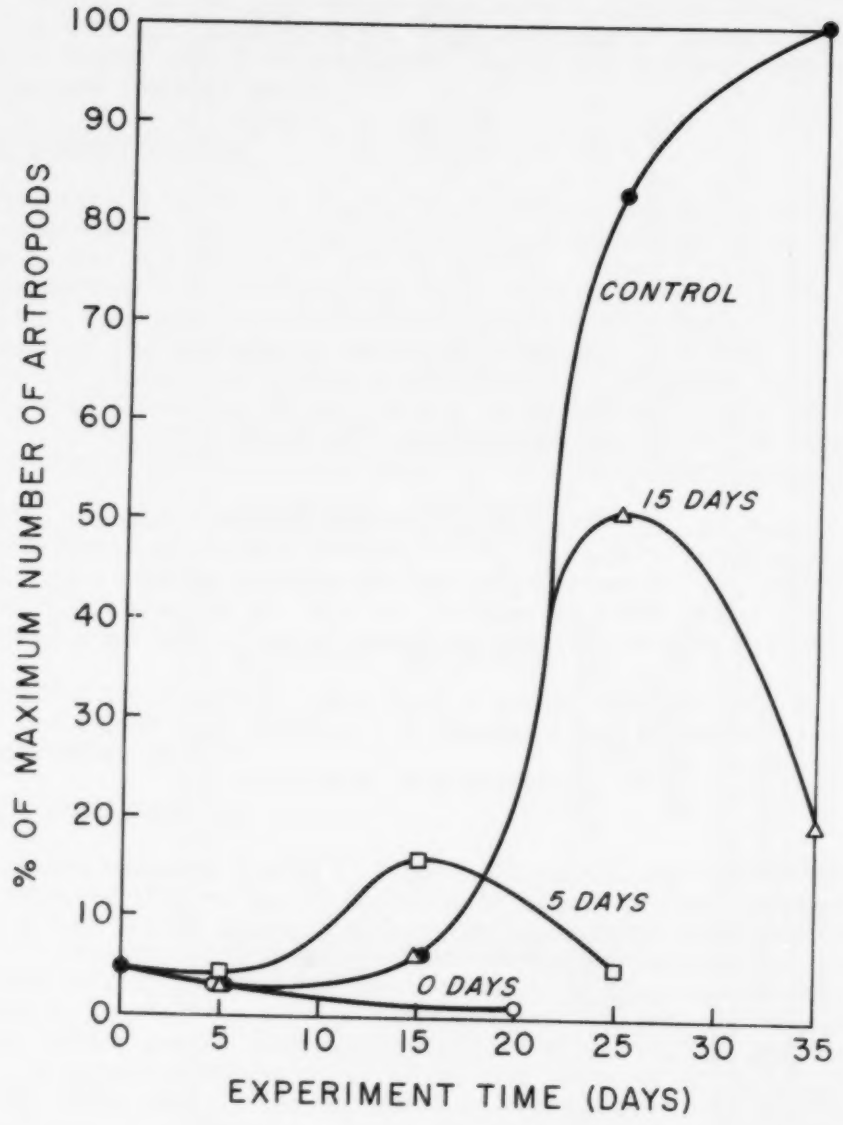

Figure 21. Survival of 0, 5 and 15 day old collembola populations in "identically" prepared coniferous forest soil/litter microcosms treated to 1500 ppm $CdCl_2$.

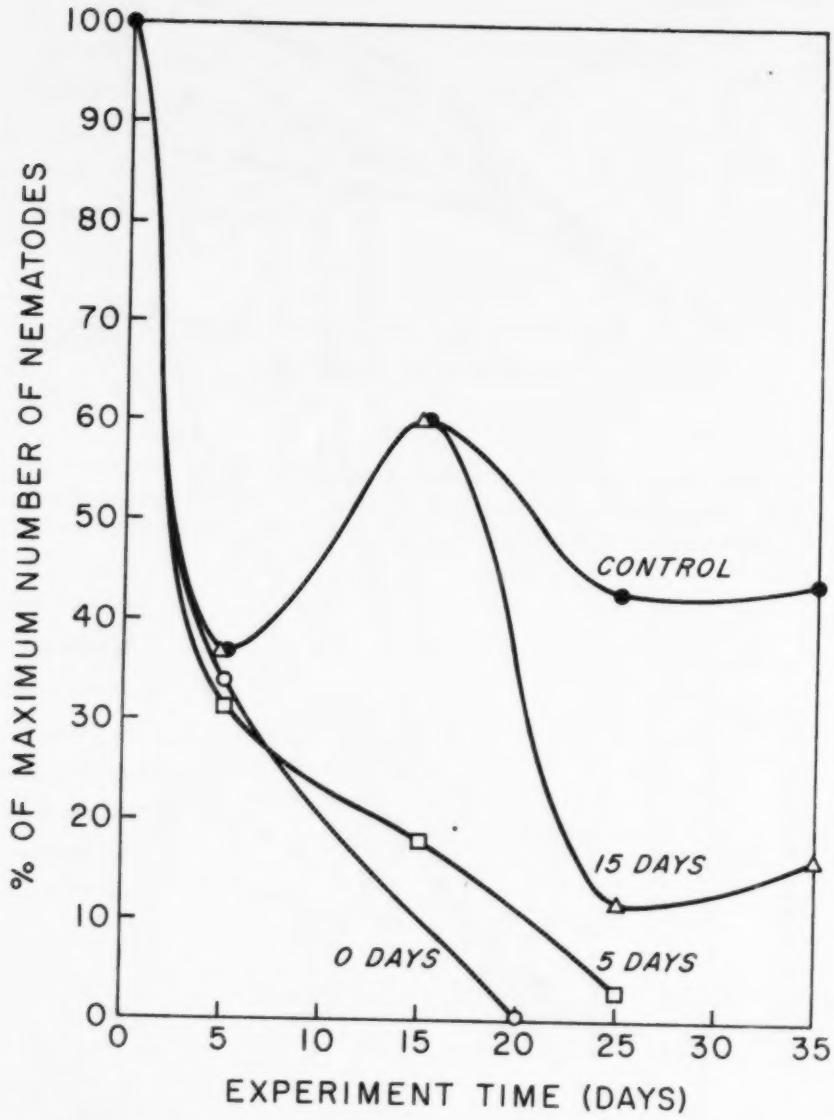

Figure 22. Survival of nematodes in 0, 5, and 15 day old "identically" prepared coniferous forest soil/litter microcosms treated to 1500 ppm $CdCl_2$.

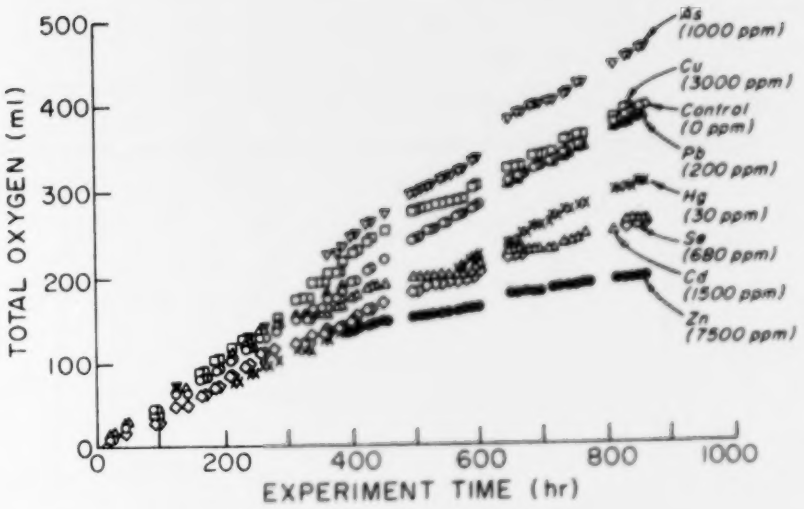

Figure 23. Accumulated oxygen consumed through time in "identically" prepared and incubated coniferous forest soil/litter microcosms treated to a mean final concentration of the indicated heavy metals (as salts).

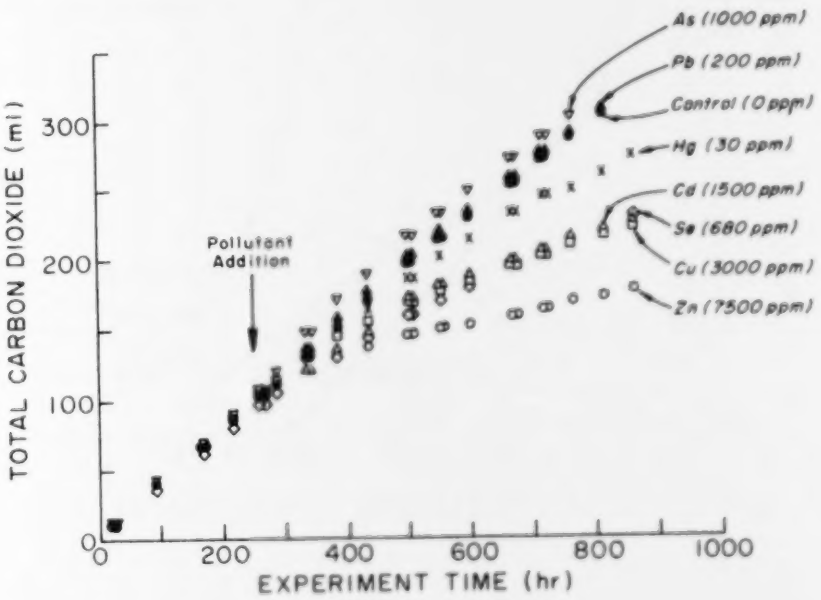

Figure 24. Accumulated carbon dioxide produced through time in "identically" prepared and incubated coniferous forest soil/litter microcosms treated to a mean final concentration of the indicated heavy metals (as salts).

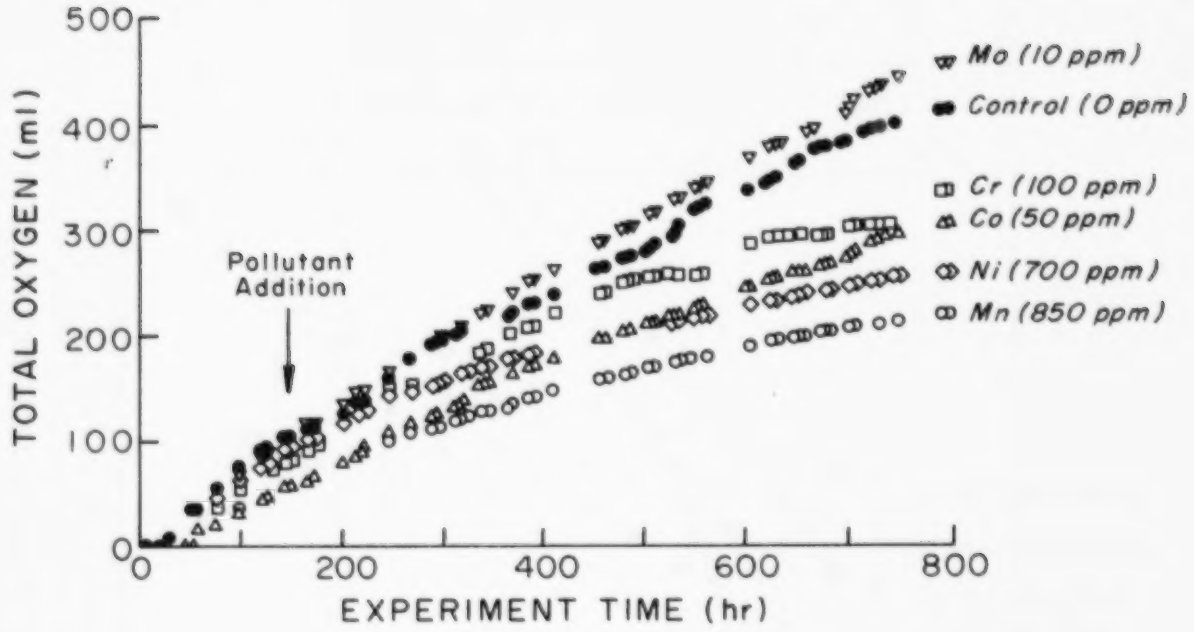

Figure 25. Accumulated oxygen consumed through time in "identically" prepared and incubated microcosms treated with the indicated trace elements.

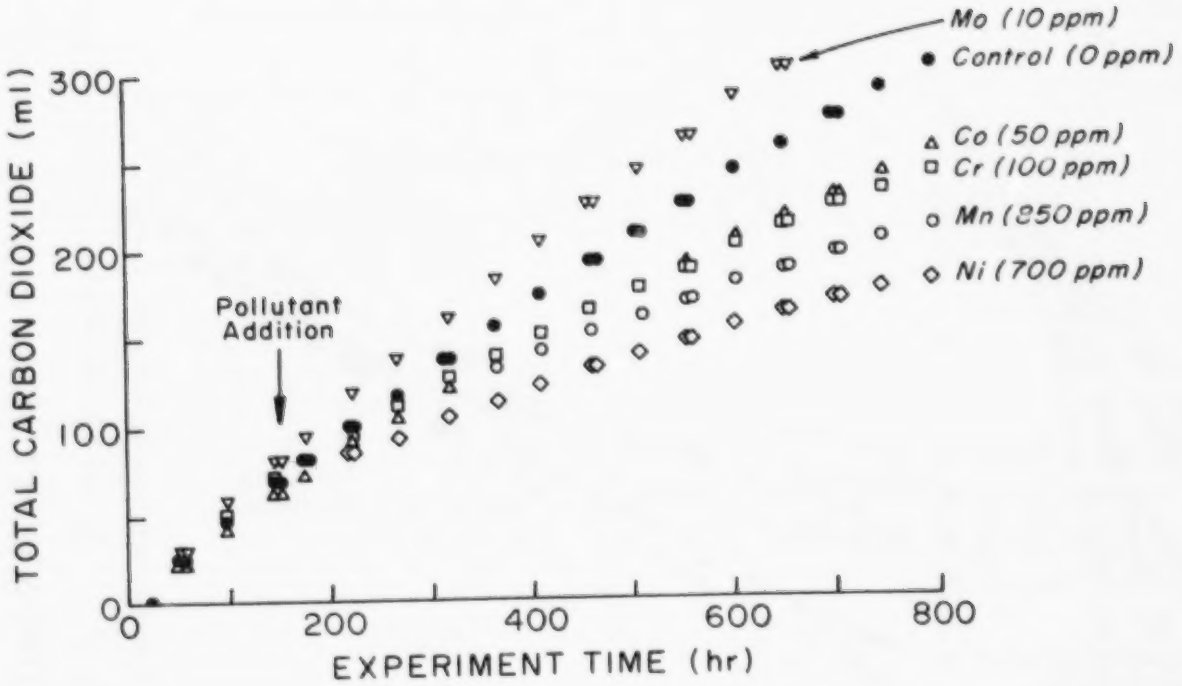

Figure 26. Accumulated CO_2 produced through time in "identically" prepared and incubated microcosms treated with the indicated trace elements.

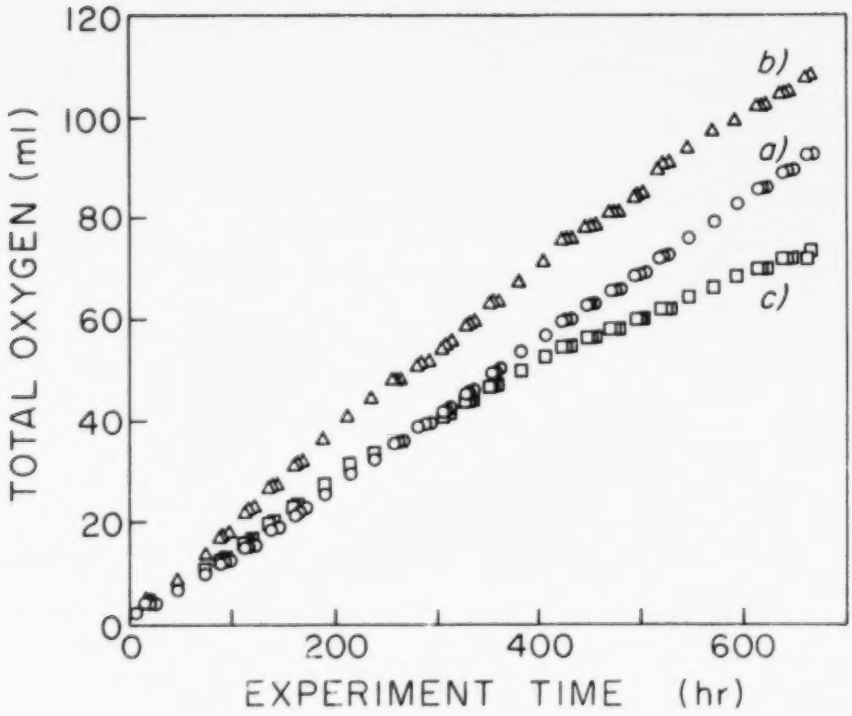

Figure 27. Accumulated oxygen consumed through time in three "identically" prepared and incubated coniferous forest microcosms treated to a final mean concentration of (a) 0.0 ppm, (b) 0.01 ppm, (c) 10.0 ppm cadmium chloride.

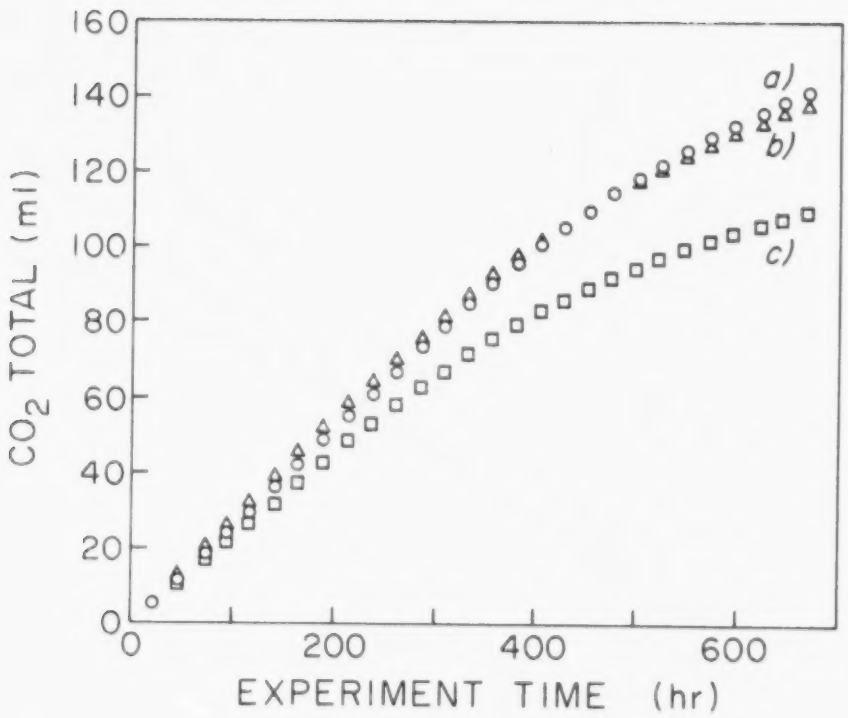

Figure 28. Accumulated carbon dioxide produced through time in three "identically" prepared and incubated coniferous forest microcosms treated to a final mean concentration of (a) 0.0 ppm, (b) 0.01 ppm, and (c) 10.0 ppm cadmium chloride.

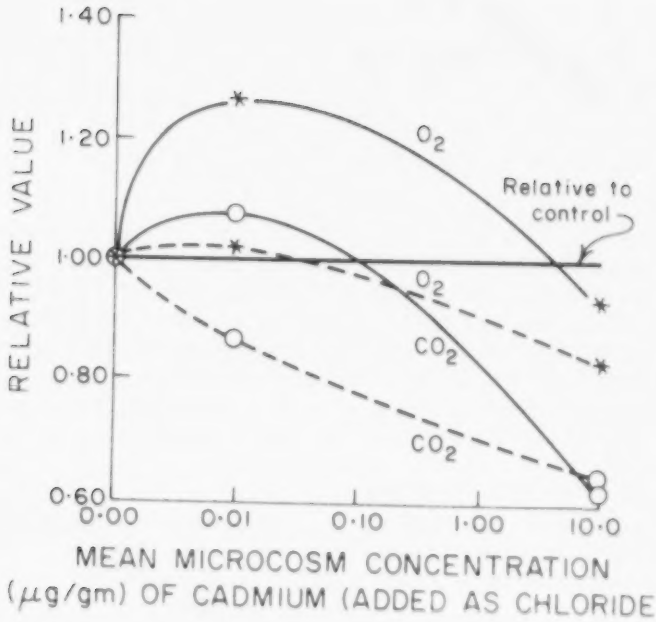

Figure 29. Graph of the relative oxygen consumption (*), and carbon dioxide generation (0) rate values for the initial 200 (solid lines), and subsequent (dashed lines) hours after cadmium addition (mean microcosm concentration) to Douglas fir soil/litter microcosms.

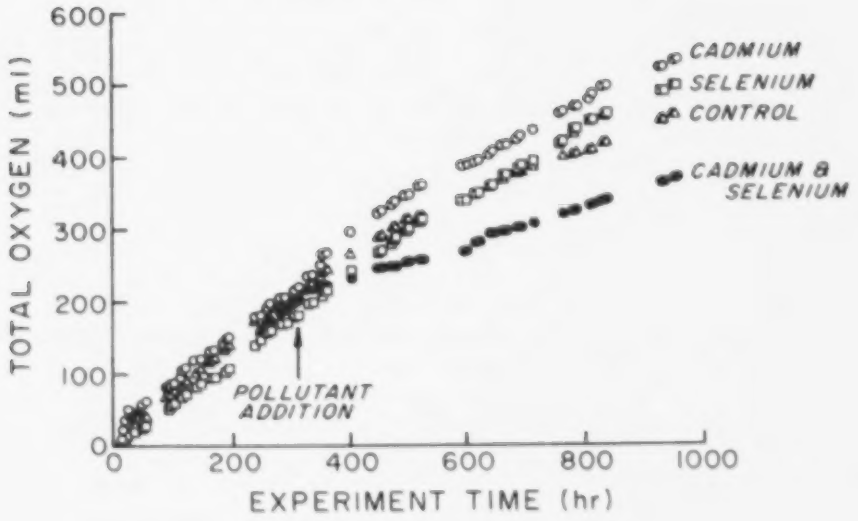

Figure 30. Accumulated oxygen consumed through time in four "identically" prepared and incubated coniferous forest microcosms treated to a mean final concentration of 0.0 ppm cadmium chloride or selenium oxide, 25 ppm cadmium chloride, 10 ppm selenium oxide, and both

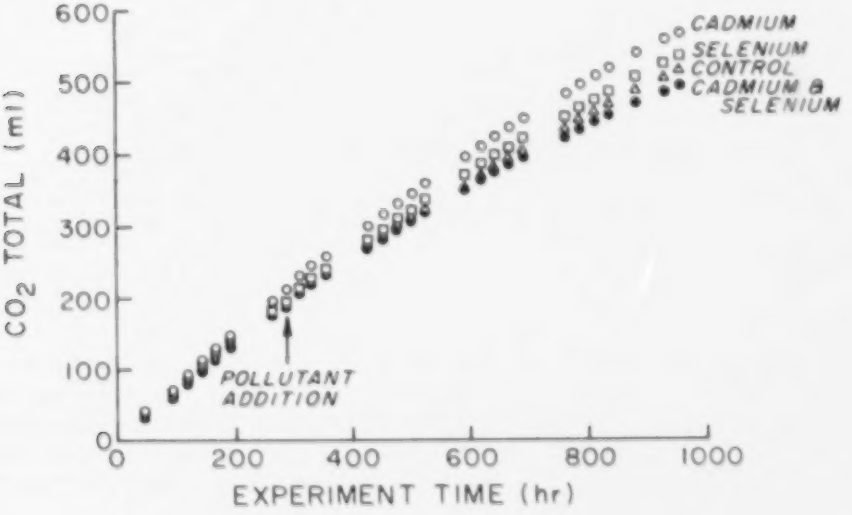

Figure 31. Accumulated carbon dioxide consumed through time in four "identically" prepared and incubated coniferous forest microcosms treated to a mean final concentration of 0.0 ppm cadmium chloride or selenium oxide, 25 ppm cadmium chloride, 10 ppm selenium oxide, and both.

4. Trace Element Combinations.

In the combined selenium/cadmium experiment, as with the high cadmium at three levels experiment (Section III. B.3), there was a delay of several hundred hours before an inhibitory effect on oxygen consumption was observed (Table 2, Figures 30 and 31). In the selenium-only treatment, effects were seen almost immediately after pollutant amendment. The interaction effect of selenium and cadmium on oxygen consumption rate was 55 percent greater than when either of the metals where added singly. The reduced respiration in the control reactor is presumably due to the inadvertent deletion of distilled water at the time of pollutant addition. Previous results (e.g., see Figures 27 and 28) indicate respiration remains relatively constant with water addition to the control microcosm.

The point to be drawn from this experiment is that combinations of trace elements may well have unexpectedly severe effects on soil decomposer processes!

C. Interpretation

In lieu of contamination of natural study sites, pollutant materials research in microcosms appears to be an acceptable alternative. From a statistical point of view, the so-called "integrator-indicators" of respiration, i.e., oxygen consumption and particularly carbon dioxide reduction, allow detection of significant treatment effects in microcosms with reasonable precision, e.g., 10.7 and 3.9 percent coefficient of variation, respectively. Even the "differentiated-indicators," i.e., organism populations, show reasonable similarity in density through time for microcosms up to six weeks old.

The similarity between microcosms is dependent upon careful attention paid to detail in microcosm preparation. A battery of inserts are prepared simultaneously. All manipulations that can be carried out with the whole homogenous substrate(s) are performed before microcosm vessel preparation! Subsequent treatments are as alike as possible!

Microcosms treated to natural variables respond respirometrically and organismally similar to what is thought to occur in nature. The effect of moisture reduction from 60 to 10 percent of field water holding capacity both reduces respiration dramatically and shifts the microcosm population from the less xerophilic bacteria to the more xerophilic fungi. The drier conditions also selected for a more saccharolytic-like microflora.

As also might be expected, increased salt treatment of the microcosm alters respiration. Both quality of salts, e.g., Cl^- or $SO_4^=$, and quantity affect respiration. From our experiments with coniferous forest soil and litter, the chloride ion is significantly more toxic than sulfate ion; this toxicity increases from 18 to 2400 ppm. The relationship of toxicity to concentration appears to be logarithmic.

Finally, the successional quality of the microcosm immediately after preparation also approximates what might be thought to occur after disturbance in nature by a natural phenomenon. Thus, there is a marked flush of gases

(O_2 and CO_2) immediately after microcosm preparation which is due to the rapid growth of the populations, particularly microorganisms, utilizing nutrients liberated from previously inaccessible microsites in the soil, and those liberated from organisms damaged during the soil manipulation process.

The respective effects of trace element pollution on the forest soil/litter microcosms is a function of at least their quality, quantity, and combinations. What the salt effects of the added pollutants are on respiration is problematical but is likely to be significant as indicated by our measurements.

Most of our trace element studies showed respiratory effects at concentrations found in nature, e.g., Cd, Cu, Se, Hg, Zn, Ni, Cr, Co, Mn, V, Li, La, and Ag. Cadmium, the only element tested so far, showed an Arndt-Schulz Law effect in that a stimulatory effect on respiration was seen at low concentrations and inhibition at high concentration.

Combinations, such as Cd and Se show a greater than added inhibitory effect when compared to the individual elements. The mechanism of this effect is unknown.

IV. Future Research

The survey of soil pollution using the microcosms will continue. Levels and combinations of materials will be tested in the near future. In order to identify specific soil ecosystem mechanisms an artificial soil system with known biological and chemical constituents is to be prepared and eventually used in the microcosm test system. The test system will also act as the prototype for a predictive computer simulation model that will be used to test for differences between predicted and observed microcosm treatment results.

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VII. Appendices

A. Microcosm Preparation

B. Inter-Experiment Maintenance Schedule

C. Intra-Experiment Maintenance Schedule

D. Scale Drawings of "Life-Support" System and Parts List

E. Electronic Control Systems

Appendix A

Microcosm Preparation

Soil and litter preparations for use in pollution effect studies have been standardized to eliminate as many variables as possible within and between experiments. This includes both handling and processing methodologies as well as microcosm insert preparation. The descriptive phases set forth below include collection and pre-preparation, soil and litter processing, insert preparation and examples concerning soil/litter moisture adjustment and pollutant spiking information. Simplified flow-diagrams indicate the various operations.

Collection and Pre-preparation--

Fresh soil and litter samples are placed in separate plastic bags and transported into the laboratory where they are spread on paper and air-dried for 24-48 hours. During this period they are stirred several times and twigs, stones, roots, and other undesirable materials removed.

Soil and Litter Processing--

After drying the soil is homogenized in batches in a Waring Blender set at low speed with intermittent removal of the blender cannister which is shaken to dislodge large soil clods and insure good mixing. Each batch is homogenized ≈ 3 minutes. Homogenized soil is collected in a large pan, and is sieved through a 1/10" mesh hand held sieve to remove larger lumps and/or other debris. The sieved soil is then hand-mixed by stirring to achieve the final homogenate.

Thin layered subsamples of this soil homogenate (≈ 1 g) are oven-dried at 110°C for 1 hour to determine the moisture content. The moisture content is then adjusted upward to attain ≈ 60% field water holding capacity (FWHC) by addition of distilled water, delivered in a fine mist (chromatograph sprayer or other suitable apparatus) while stirring. The wetted soil is allowed to equilibrate 2 hours and moisture determinations are repeated to assure proper moisture content. Subsamples may also be taken for pH determinations or other analyses if desired.

Enough soil is prepared, as described above, to make up a predetermined number of microcosm inserts within 24 hours after the initial homogenizing operation. Microcosms are assembled on the same day as the water addition.

After drying, the litter (coniferous) is sieved in a rotating drum sifter with 16 mesh screening (modified rock polishing apparatus) and collected in a common container and homogenized with hand stirring. Thin layer subsamples

(≈ 0.5 g) are oven-dried at 110°C for 1 hour to determine the moisture content. The moisture content is then adjusted upward in the same manner as soils are wetted to attain ≈ 78% moisture content by weight. After settling 2 hours moisture determinations are again made to confirm that the desired level has been achieved. At this point subsamples may be taken for pH determinations and/or other analyses.

Enough litter is prepared in this manner to make up the desired number of microcosm inserts on the same day on which it was wetted.

Insert Preparation --

Berzelius beaker inserts, pretared and numbered, are placed in a circular configuration on paper or on a large tray. The prepared soil is then poured onto a 3' x 3' wooden framed screen (1/4" mesh screen overlaying a 1/8" mesh screen) and sieved into the inserts by shaking the screen in a reciprocating manner until each insert contains approximately 150 g of soil. The inserts are then individually weighed and the amount of soil is adjusted to the exact amount, 150 g (wet weight) per insert. To standardize the interstitial pore space the soil is then compressed by placing a snug fitting wooden cylinder into the beaker and carefully placing a 12 kg weight (lead brick) on top of the cylinder for one minute. The compression apparatus is then removed and the prepared litter (15 g, wet weight) is added to the top of the soil, spread to attain even distribution and the compression operation repeated.

Immediately after removing the compression apparatus a 4" x 4" piece of Teflon film is placed over the top of the insert followed by a 4½" x 4½" piece of Saran wrap with a 0.5 cm diameter central perforation. These two top layers are secured with a rubber band. Studies indicate that this cover arrangement allows for adequate aeration and cuts moisture loss from the system to <0.2 ml/day under incubation conditions.

The microcosm inserts are then placed into an incubator at the desired temperature (20°C) for a 10-14 day equilibrating period prior to use in the SER system.

Water loss from the inserts is replaced each week by adding distilled water drop-wise over the litter surface until the original weight is attained.

Soil/litter Moisture Adjustment--

The optimal moisture content for an experimental microcosm is approximately 60-78% of the field water holding capacity (FWHC) of the soil and litter. This was found to be 18-23% moisture for soil and 48-63% moisture for litter, as measured by drying wet samples 20 hours at 110°C.

Determining final weight of soil:

ex. 1: If 100 g final weight of remoistened soil is needed, the amount of water present would be 23 g per 77 g dry soil.

$\frac{23\ g}{77\ g}$ = 0.299 which is the proportion of water to dry soil at 78% FWHC.

ex. 2: The same applies to the litter: therefore in 100 g final weight sample there are 63 g water and 37 g dry litter.

$\frac{63\ g}{37\ g}$ = 1.703 which is the proportion of water to dry litter at 78% FWHC.

Microcosms are analyzed on a dry weight basis, and dry weight is the basis for microcosm preparation.

ex. 3: If 15 g dry weight soil is needed, the amount of water to be added is:

$\frac{x}{15\ g}$ = 0.299; x = 4.485 g water.

4.485 g water + 15 g dry soil = 19.485 g final weight.

ex. 4: If 45 g dry weight litter is needed, the amount of water to be added is:

$\frac{x}{45\ g}$ = 1.703; x = 76.635 g water.

76.635 g water + 45 g dry litter = 121.635 g final weight.

Pollutant Addition to Microcosms--

As previously described in this report an assessment of the effects of certain trace elements (e.g., heavy metals) on decomposition was accomplished. In this regard certain methods and techniques were devised and used to standardize the addition to or spiking of microcosm inserts with aqueous solutions of the desired pollutant.

The following information describes the preparation of spiking solutions, the time of spiking the inserts and the pollutant application technique.

Spiking solutions were prepared by accurately weighing out and solubilizing the selected pollutant (e.g., $CdCl_2$) in distilled deionized water. An example of the calculations to attain a given concentration per a given dry weight of soil follows:

Basic Formula:

$$1.\ \frac{\text{Formula Wt. of Compound}}{\text{(Atomic Weight Element)}} \times \text{µg Element/g of Dry Wt. of Soil or Litter Needed} \times \text{Wt. of dry soil}^{*}$$

Diagram 1

SOIL/LITTER COLLECTION, PREPARATION AND PROCESSING

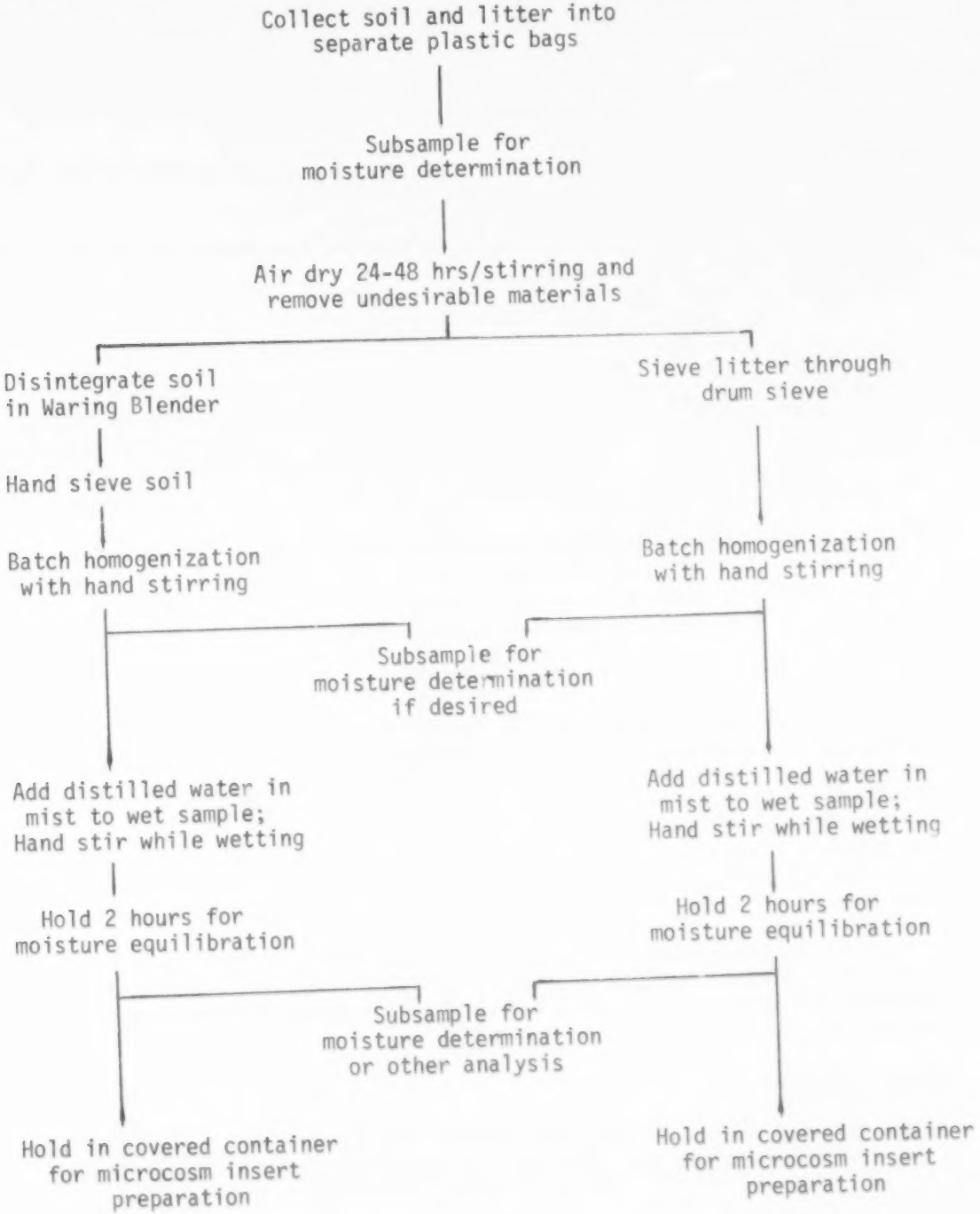
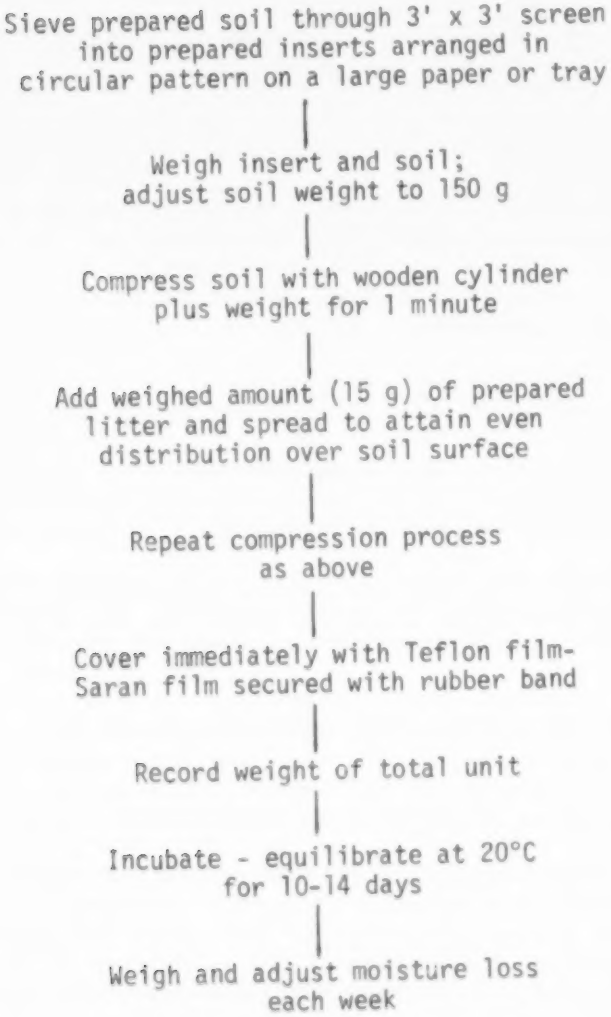

Collect soil and litter into separate plastic bags
Subsample for moisture determination
Air dry 24-48 hrs/stirring and remove undesirable materials
Disintegrate soil in Waring Blender
Hand sieve soil
Batch homogenization with hand stirring
Sieve litter through drum sieve
Batch homogenization with hand stirring
Subsample for moisture determination if desired
Add distilled water in mist to wet sample; Hand stir while wetting
Hold 2 hours for moisture equilibration
Add distilled water in mist to wet sample; Hand stir while wetting
Hold 2 hours for moisture equilibration
Subsample for moisture determination or other analysis
Hold in covered container for microcosm insert preparation
Hold in covered container for microcosm insert preparation

Diagram 2

MICROCOSM INSERT ASSEMBLY


Sieve prepared soil through 3' x 3' screen into prepared inserts arranged in circular pattern on a large paper or tray
Weigh insert and soil; adjust soil weight to 150 g
Compress soil with wooden cylinder plus weight for 1 minute
Add weighed amount (15 g) of prepared litter and spread to attain even distribution over soil surface
Repeat compression process as above
Cover immediately with Teflon film-Saran film secured with rubber band
Record weight of total unit
Incubate - equilibrate at 20°C for 10-14 days
Weigh and adjust moisture loss each week

= number of grams of the compound needed per horizon.

* Soil or litter, a separate calculation for each.

2. $\frac{\text{(Gram compound per horizon) x (Vol. solution prepared)}}{\text{(total vol. delivered per horizon)}}$

= the number of grams compound needed to prepare the solution.

ex. 1: Wanted: 1000 ppm of Cd as $CdCl_2$ in 100 g dry soil, will spike the horizon with 4 ml of the 20 ml of spike solution prepared.

Using Formula 1:

$$\frac{(183.4 \text{ g } CdCl_2)(10^{-3} \text{ parts})(100 \text{ g})}{(112.4 \text{ g Cd})} = 0.163 \text{ g}$$

Using Formula 2:

$$\frac{(0.163 \text{ g})(20 \text{ ml})}{4 \text{ ml}} = 0.815 \text{ g.}$$

Solution concentrations were such that when 4 ml of solution was added to the soil horizon and 2 ml added to the litter horizon of each insert the final mean concentration desired was attained.

After the inserts have been monitored in the reactor module for 10 days to determine background values of oxygen consumed, CO_2 produced and heat energy flux they are removed one at a time from the module and the pollutant is added.

The pollutant, in aqueous form, is delivered via 1 ml, discardable tuberculin syringes with 2"-2½", 22 gauge luer-loc needles. A small portion of neoprene rubber is pierced by the needle and serves as a depth marker when injecting. A disc (3 mm thick) of transparent plastic with 45 holes at 1 cm square grid points is placed on top of the litter horizon. The needle is inserted through these grid holes and the pollutant injected by delivering an equal portion through each hole until 2 ml are delivered at each level. In this manner 2 ml is placed approximately 3/4" above the bottom of the soil layer, 2 ml just beneath the surface of the soil layer (1 cm) and 2 ml of the appropriate concentration into the center or midway into the litter horizon.

As soon as spiking is completed the insert is returned to the reactor module.

Appendix B

Inter-Experiment Maintenance Schedule

SER Calibration--

The SER units are calibrated to determine the heat demand and loss of each unit. A heat control is run with each experiment to ascertain heating fluctuations which occur in a non respiring SER. A beaker containing 500 g of dry compressed soil is placed in the SER heat control during the actual SER experiments since the microcosms in the test reactors act as insulation and alter the heat demand from that of empty dewar flasks.

Calibration Check List--

1. SERs are readied for operation.
   a. Reactor moducles are dried.
   b. The manometer fluid is adjusted to a medial position.
   c. Agar bridge integrity is checked.
   d. H_2 and O_2 electrodes are inspected for deposits, breakage or electrolyte leaks.
   e. NaOH sample port (valve) is lubricated and rubber septums replaced.
   f. Stopcocks and ground glass joints are lubricated.
   g. Air filters are checked for excessive liquid absorption.
2. Starting SERs.
   a. Main voltage for gas generation is set between 12.5 and 15.5 volts DC, depending on total unit load on the power supply. Power on.
   b. Individual panel voltages are set at approximately 13.0 volts AC, depending on heating element resistance. Power on.
   c. Heat time meters are set to zero. Power on.
   d. SER lids are lowered and tightened shut.

e. Electrolyte level in O_2 probe is set even with O_2 generator electrolyte level.

f. All thermometers (thermoregulators) are set for 20°C.

3. Data collection.

a. Computer data sheets are used for collecting and recording all SER data.

b. Readings are made three times daily, at approximately 08:30, 12:00, and 16:00. One reading is made on weekends.

c. Readings consist of recording barometric pressure, room temperature, manometer level, time, date, calibration number, electrolyte level in O_2 probe, heating times, and panel voltages for the individual units are recorded, then reset at each reading to the original setting of 13.00 ± 0.08 volts AC.

d. Amends are made in the apparatus, if any unusual readings or activity is observed i.e., air leaks causing gas generation.

Duration of Calibration--

Calibration runs were originally made prior to and after each experiment. Currently, calibration runs are not being made this frequently since heat demands appear to be linear for each unit. A calibration run extends from three to seven days depending on the needs of the system.

Trouble Shooting the System and Agar Bridge Preparation--

Certain symptoms have been found to be indicative of the cause of minor malfunctions within the SER system. These have been tabulated (Table 6) to aid in correcting these problems. Instructions are provided if it is necessary to find small leaks by submersion of the reactor module and/or to prepare agar bridges.

1. Submerging a SER: This is done when the SER is empty (i.e., without a microcosm).

a. The manometer is replaced by a stopcock and securely tied, with the stopcock open to allow air flow.

b. The lid is tightened down evenly with the wing nuts.

c. The 3-way stopcock is closed to the atmosphere.

d. The O_2 probe is lifted out of the O_2 generator. A meter length of tubing is placed over the 3-way stopcock. The stopcock is opened and air expelled into the unit, then the tubing is clamped shut.

TABLE 6. TROUBLE SHOOTING

Symptom(s)	Possible Cause(s)	Remedy(s)
1. spontaneous or over generation of H_2 and O_2	1. a. lid not sealed tightly	1. a. tighten all three wing nuts on lid evenly
	b. inadequate tension at joints	b. put springs or rubber bands snuggly around the joint
	c. stopcock(s) open	c. check positions of all stopcocks
	d. ground glass joints not adequately lubricated	d. lubricate stiff joints
	e. CO_2 valve leak	e. regrease valve. If necessary, submerge the unit in a bath of water. Look for air bubbles around the CO_2 valve. If air is leaking at the valve/lid contact, re-epoxy into place.
	f. hole in buret bulb	f. pressurize buret and submerge in a water bath: If air bubbles form on bulb, replace.
	g. tygon tubing/glass connectors loose	g. look for air bubbles between glass and tubing. If loose, replace tubing or add a hose clamp, tighten snuggly around the joint.
2. under generation of H_2 and O_2, or a delayed response between pressure changes in the flask and gas production	2. a. blocked air line	2. a. clean stopcock openings of excess lubricant.
	b. all items listed in 1 (a-g).	b. see 1 (a-g).
3. not generating gases (H_2, O_2) or the unit not heating properly	3. Electrical circuit or electronics panels malfunctioning	3. a. check position of alligator clips and tighten or replace if corroded
	a. alligator clip off of electode	b. check all switches on panel and indicator light
	b. panel switch off	c. tighten panel in position, clean contact strips; try replacement panel
	c. panel malfunction	d. check for loose wires, check terminals
	d. wire broken or corroded	e. if probes are blackened, drain electrolyte, rinse with distilled water. Clean electrodes with 50% nitric acid (aq.) then let set 15-30 min. Drain and rinse 6-12 times with distilled water to remove all traces of acid. Refill with fresh electrolyte. If electrodes are loose, epoxy in place.
	e. electrodes coated or loose	f. replace electrolyte
	f. electrolyte contaminated	
4. discoloration of agar bridge and/or electrolyte	4. a. microbial growth	4. a. if the discoloration is gold-green-brown; remove bridge and remove agar. Rinse with 50% nitric acid (aq). Reform bridge using an anti-bacterial agent.
	b. impurity in Na_2SO_4 reagent, agar or distilled water	b. prepare fresh electrolyte or agar from new reagent stock, use distilled water direct from source.
	c. chemical reaction with the contents of the SER	c. replace air filters in H-tube.

TABLE 6. (CON'T.)

Symptom(s)	Possible Cause(s)	Remedy(s)
5. manometer fluid loosing color	5. pH change	5. replace air filters in U-tube. Acidify manometer fluid below pH 4.
6. "water" in air line	6. a. evaporation or condensation from generators, manometer fluid, microcosms, or NaOH.	6. a. replace air filters, dry the lines with an air jet. Make certain that chamber and SER temperatures do not fluctuate.
	b. inadequate gas production i.e. gas production is not meeting the demands of the microcosm Electrolyte is backing up the O_2 electrode and into the air line.	b. electrolyte level should be readjusted. Filters changed if wet. May need to remove the microcosm if gas generating capacity is exceeded. Gas generation can be increased by increasing power supply voltage output; do not exceed 100 ma total capacity with all units generating with a nine unit lead, maximum voltage setting is theoretically 31.0 V DC.

e. The SER is submerged in a water bath, first allowing trapped air to escape from under the stand and around the lid edge and valves.

f. The unit is carefully observed for several minutes, to detect air bubble formation.

g. The source of any air leak is noted and sealed.

2. Forming agar bridges.

a. Five percent Noble agar is prepared by placing 5 g Noble agar in 95 ml distilled water. The solution is heated and stirred until dissolved.

b. The clean glass fritted tubing is heated, then placed in the agar for 5 minutes to allow the agar to permeate the frit. Gentle vacuum is applied to draw the agar about 3 cm into the tubing. Then gentle air pressure is applied to force the agar back into the pores, until the agar is 2 cm above the frit.

c. The bridge is removed from the agar and dried 10 minutes, then dipped into the agar to coat the outer surface. The bridge is allowed to set 10 minutes then placed in the electrolyte (8% Na_2SO_4 aq. with 0.05% NaN_3), until it is replaced in the SER.

Appendix C

Intra-Experiment Maintenance Schedule

Experiment Initiation--

At the start of a SER experiment the microcosms are placed inside the SER units and data are collected from the respiration of these microcosms. The experiment itself is affixed to the end of the calibration run or unit warm-up period. All units must be showing proper heating, maintenance of voltage settings, the ability to produce oxygen gas and no contamination of the electrolyte or agar bridge, or air leakage in any part of the sealed system.

Microcosm inserts are placed in the reactor modules after the morning set of readings for the calibration run. This ends the calibration period. The collection of experimental data begins at the noon set of readings. This allows the microcosms and SERs to equilibrate for 2-3 hours.

SER Experiment Initiation Procedures--

1. The usual morning calibration readings are taken.

2. Microcosms are brought two at a time from the incubator to the SER incubation chamber.

3. The unit is opened and the microcosm placed inside with the Teflon and Saran covering removed.

4. The lid is placed on the reactor module, but not tightened down. Ten ml of 0.6 N NaOH* (aq.) is added to the NaOH cup via the port on the SER cover. The SER lid is then secured. Two NaOH blanks are taken at the beginning of the experiment and at the time when samples are withdrawn.

5. The manometer fluid is set to a medial position, the electrolyte level in the O_2 electrode is set even with the electrolyte level in the O_2 generator.

6. At the time of the noon readings the following adjustments are made.

---

* The standardized 0.6 N NaOH solution must be made up with CO_2-free distilled deionized water and maintained in a sealed container to obtain the true CO_2 value of CO_2 produced and to obtain accurate titration values.

a. The tension on the lid thumbscrewsare balanced and they are retightened.

b. Barometric pressure, room and unit temperatures, manometric pressure, time, date, experiment number, and electrolyte level in the O_2 electrode are all recorded.

c. The clock meters are all shut off and set to zero, then power is turned on again.

d. Panel voltages are set to their individual settings.

e. Electrolyte level is set to zero in the buret or at maximum capacity in the buret bulb of the hydrogen module.

7. O_2 and other readings indicated in 6b above are made at noon each day. Electrolyte level is reset in the hydrogen module.

8. NaOH samples (CO_2 trapping agent) are collected 24 hours after the beginning of the experiment. After this, all other samples are collected at 48 hour intervals. The O_2 and CO_2 readings are made during the noon data collection.

9. The readings are made three times daily and once on weekends. The standard readings consist of those items listed in 6b along with listing clock meter readings, panel voltage readings and reset, plus 7 and 8 when applicable.

10. SERs are constantly observed at the time of data collection for any malfunction. These are repaired immediately.

Duration of an Experiment--

SER experiments are run for approximately 31-35 days. The first 10-14 days after placement of the microcosms in the SERs is the internal control period in which the microcosms equilibrate to a relatively constant level of respiratory activity. The microcosms can then be spiked with pollutants. Post spike period run time is 21 days.

Appendix D

Scale Drawings of "Life Support" System and Parts List

Figures 32-37 show the various modules and component parts of the "life support" unit including the hook-up of the total system. Also included is a tabulation of the parts with identifying information, procurement sources and approximate costs (Table 7).

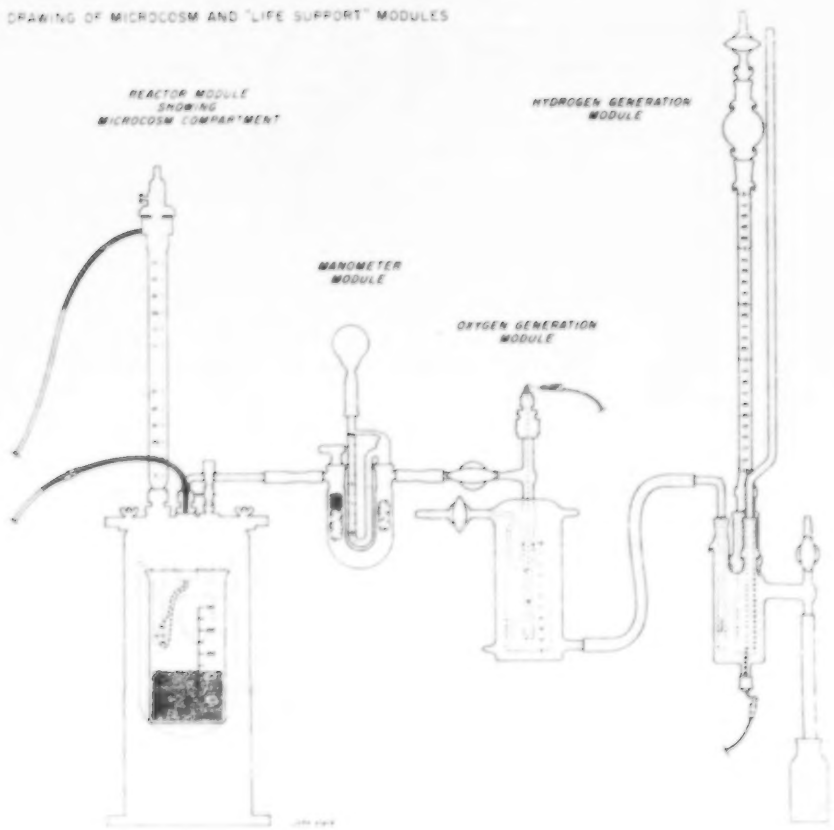

DRAWING OF MICROCOSM AND "LIFE SUPPORT" MODULES
REACTOR MODULE SHOWING MICROCOSM COMPARTMENT
HYDROGEN GENERATION MODULE
MANOMETER MODULE
OXYGEN GENERATION MODULE

Figure 32. Drawing of microcosm "life support" system including reactor, pressure monitoring, O_2 generating and H_2 generating module.

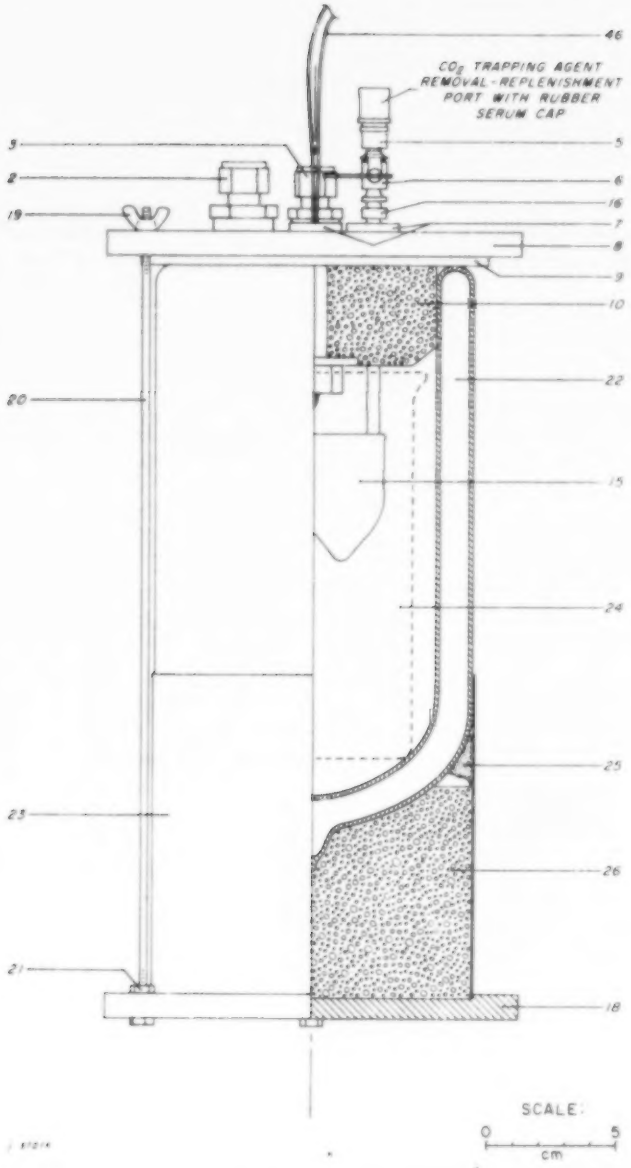


Figure 33. Cut-away view of respirator reactor module.

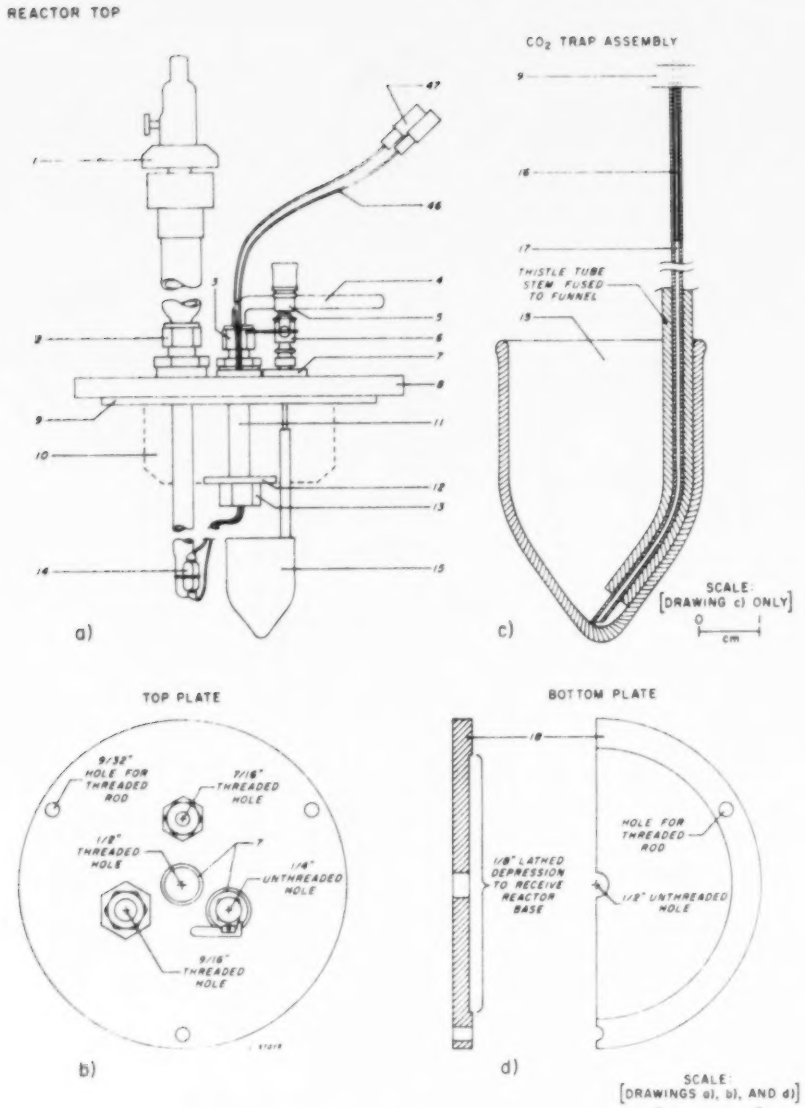


Figure 34. Schematic diagram of reactor module components showing (a) lateral view of top assembly, (b) top view of top plate, (c) lateral view of CO_2 trap assembly, and (d) top and cut-away side view of bottom plate.

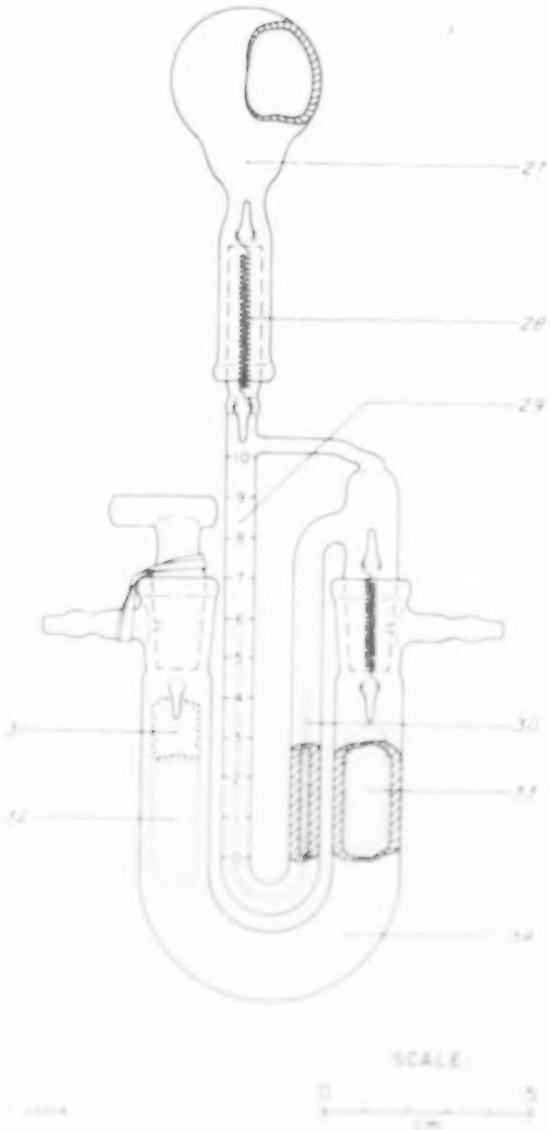


Figure 35. "U" tube pressure monitoring module.

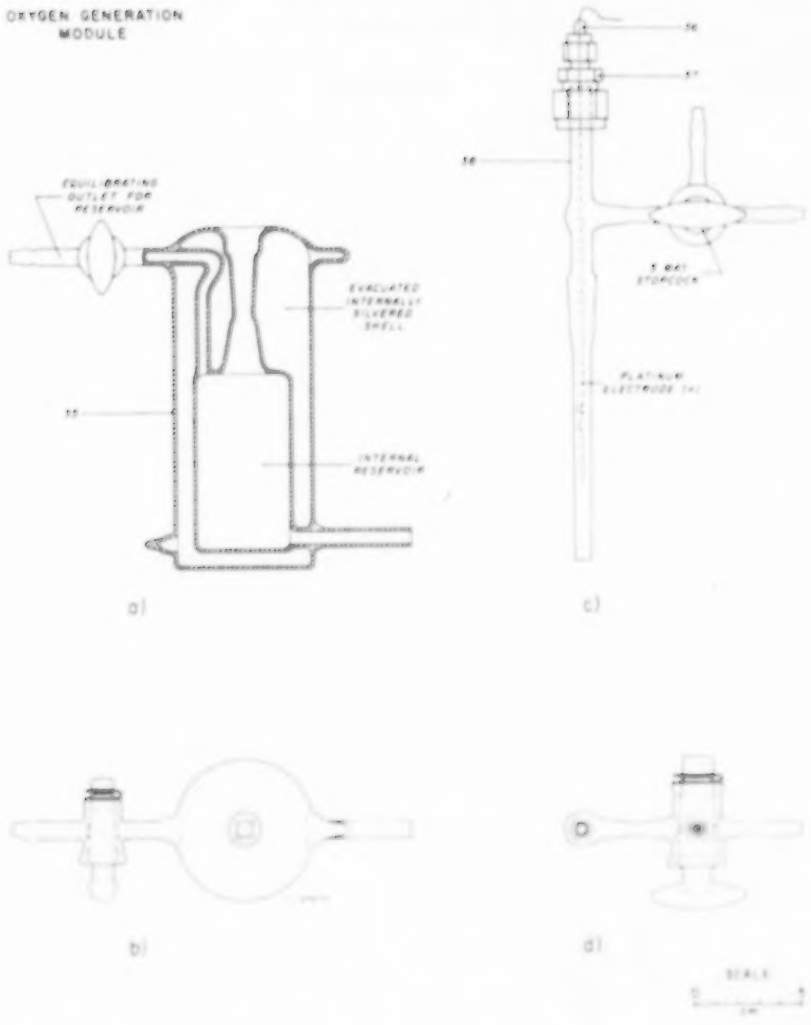


Figure 36. Oxygen generation module showing (a) cut-away view of base with evacuated/silvered external shell, (b) top view of generator module, (c) oxygen probe component with electrode and (d) top view of oxygen probe.

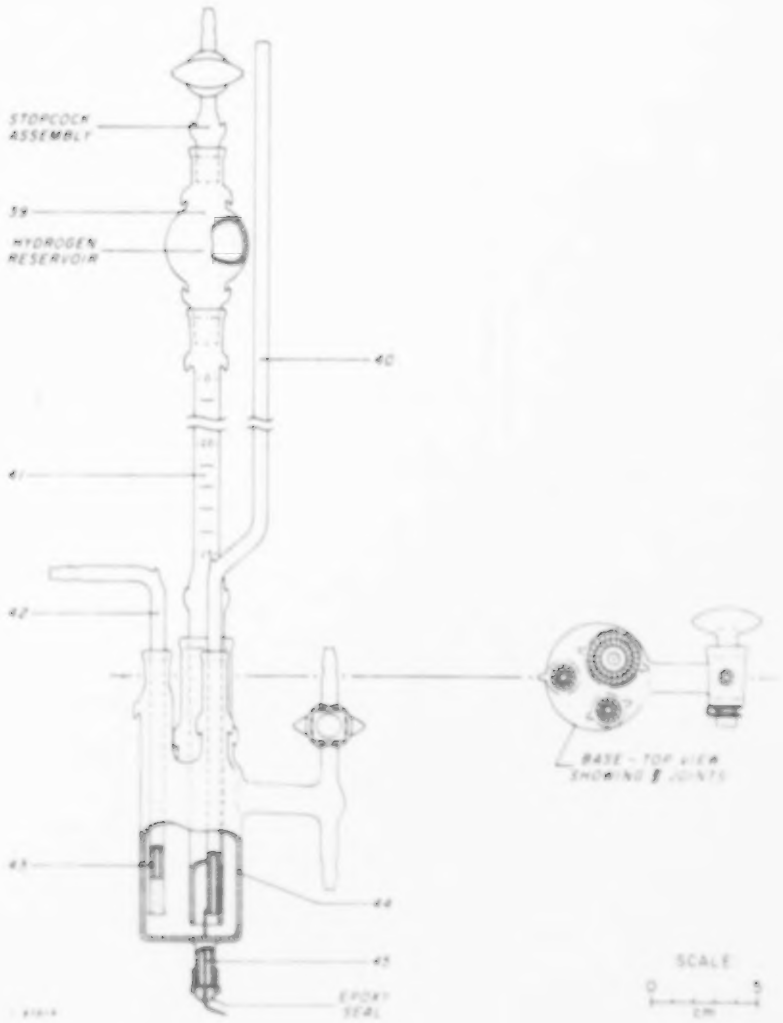


Figure 37. Lateral view of hydrogen generation module.

TABLE 7. TABULATION OF SOIL ECOSYSTEM RESPIROMETER (SER) COMPONENTS, SOURCES AND APPROXIMATE COSTS

Diagram Number	Catalog Number	Part Description	Purchase Source*	Unit Price
		I. REACTOR MODULE		
1	61845-009	Thermometer, Bromwill 0-50° C	VWR	34.00
2	NY-600-1-OR	Swaglok fittings, 9/16-18	Swaglok	3.20
3	NY-400-1-OR	Swaglok fittings, 7/16-20	Swaglok	3.10
4		Glass capillary tubing ID 1/16" OD 1/4"	VWR	.40
5	B-D #3112	BD luer lock fitting w/rubber septum	B-D	.71
6	86570	Stainless steel and nylon valve; IFLI	Hamilton	6.00
7		Aluminum tubing ID 1/2" OD 3/4", rings 1/4" high	Custom made rings	.10
8		Turned aluminum disk, 1/2" thick, 6-5/8" diameter	Custom made	4.00
9		Neoprene sheeting for gasket, 1/8" thick	Power Transmission Albany, OR	.40
10		Styrofoam inner lid (insulation)	Hobby Shop	1.00
11		Threaded 1/2" diameter nylon tubing	Hardware store	.50
12		1" nylon washer	Custom made	.25
13		1/2" brass nut	Hardware store	.20
14		10 kΩ resistors (2)	Kierulff Elect. Seattle, WA	2.00
15	46180	Thistletubes; to custom make NaOH cups	VWR	1.00
16		18 gauge x 2" med. hypodermic needle	VWR	1.50
17		Microtubing; size 050 x 090, 100 ft. roll	Norton Plastics Akron, OH	8.00
18		Turned aluminum disk, 1/2" thick with 1/8" lathed depression, 6-5/8" diameter	Custom made	4.00
19		Standard wing nut for 3/16" rod	Hardware store	.07
20		Threaded rod 3/16" diameter	Hardware store	.45
21		Lock nuts for 3/16" rod	Hardware store	.05
22	K-611420	Dewar flask, 95mm I.D., 125mm O.D., 1200ml cap.	Fontes-Martin, Evanston, IL	33.00
23		Aluminum Dewar base		
24	14020	Berzelius beakers 600 ml capacity; no spout	Elico Glass Co.	2.30
25		Tar sealing compound, Matrix Binder		
26		Polyurethane foam	Hardware	.25

TABLE 7. (CON'T.)

Diagram Number	Catalog Number	Part Description	Purchase Source*	Unit Price
		II. MANOMETER MODULE		
27		25 ml boiling flask, for manometer	Custom modified	10.00
28		Small springs	VWR	.05
29		Modified glass capillary for manometer fluid	Custom made	10.00
30		Kreb's manometer fluid, acidified	Manometric[1] Techniques	
31		Activated charcoal granules	VWR	
32		Fine textured glass wool	VWR	
33		Ozone trapping filter[2]		
34	Kimble 46050	U-tube; modified 100 mm drying tube	Kimax (VWR)	14.58
		III. OXYGEN GENERATOR MODULE		
35		Oxygen generator	Custom made	100.00
36		13 cm long platinum wire epoxyed in glass cap. tubing 3/4" long	Custom made	5.00
37	KY-810-6-4	Swaglok fittings, nylon reducing union	Swaglok	3.70
38		O_2 probe glass support	Custom made	
		IV. HYDROGEN GENERATOR MODULE		
39		Modified condensation flask; hydrogen reservoir w/stopcock	Custom made	
40		Pressure equilization standpipe	Custom made	
41		Inverted 25 ml pyrex buret	Custom made	
42		4-8 m pore size fritted glass dispersion tubes (modify) O.D. 10 mm, length 150, porosity code E-10	ACE Glass, Inc. Louisville, KY	5.35
43		Noble agar; use 5% in H_2O: electrolyte support	Difco	
44		Hydrogen generator base (cost includes modification of all glassware)	Custom made	140.00
45		Platinum anode, secured in epoxy	Custom made	
		IV. ELECTRICAL SYSTEM		
46		Single strand electical wire; 22 gauge PVC coating	Kierulff Elect. Seattle, WA	.55
47		Snap connectors, U connectors for wiring	Hardware store	.30
		Alligator clips 3/4" length	Hardware store	.10
Not Pict	382 line 2032	Minature indicator lamps 14V	GE	.65
Not Pict		Heath kit electronics converter for D.C. current to generate gases	Kierulff Elect. Seattle, WA	150.00
Not Pict	SC-240634 AAAE1	Clock meters 120V 60 Hz 2.5w	GE	42.50

[1] Manometric Techniques, Umbeit, Stauffer and Burris, Burgess Publ. Co., Minn., MN, 1964, 4th ed.

[2] Ozone Problem in Electrolyte Respirometry and its Solution, D. J. Woodland, J. Applied Ecology, 10:661-2, 1973.

TABLE 7. (CON'T.)

Diagram Number	Catalog Number	Part Description	Purchase Source*	Unit Price
		VI. MISCELLANEOUS		
48		Tygon tubing ID 1/4" OD 1/4"	VWR	
49		Electrolyte overflow bottle, 200 ml, milk dilution	VWR	.60
Not Pict	6403-20	Hose clamps with thumb screws 1/2" to 3/4"	Horizon	.92
Not Pict		Silastic sealing compound, GE RTV 108 silicone rubber adhesive sealant	GSA	
Not Pict	8040-00-159-4846	Epoxy kit (for cementing metal, glass) Devcon	Hardware	1.25
Not Pict	size 11 1/2" x 260"	Teflon sealing tape, around swaglok fittings	Lake City Industrial Products, Lake City, PA	.90
Not Pict	21639-045	Clamps for supporting units, 3 prong vinylized	VWR	3.60
Not Pict	21641-047	Clamps for supporting units, 3 prong vinylized	VWR	4.50
Not Pict	21677-000	Clamp holder, Fisher Castaloy	VWR	1.75
Not Pict		Nidibutylidithio carbamate, active ingredient in ozone trap	ICN Pharamcuticals, Inc.	
Not Pict		0.6 N NaOH (aq.) CO_2 absorbent	VWR	
Not Pict		8% Na_2SO_4 (aq.) electrolyte	VWR	
Not Pict	970 V	Low/high vacuum/pressure Dow Corning silicone grease	VWR	

* Where appropriate

Appendix E

Electronic Control System

The following schematic (Fig. 38) depicts the electronics control system for the "life support" unit. Electronic components, procurement sources and approximate costs are incorporated in Table 7, Appendix D.

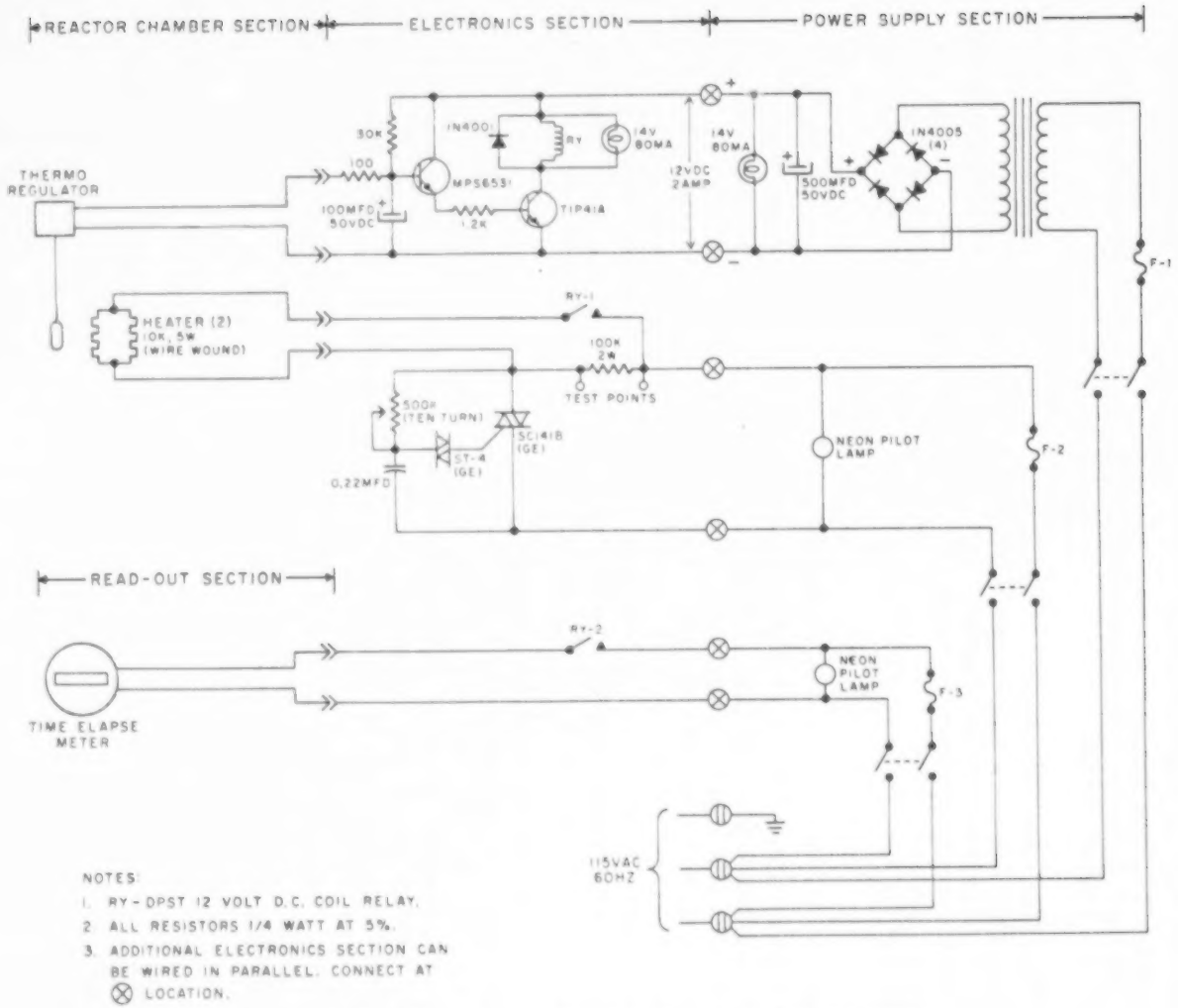


Figure 38. Microcosm electronic circuit diagram.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

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EPA-600/3-77-091		

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	6. PERFORMING ORGANIZATION CODE

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15. SUPPLEMENTARY NOTES

16. ABSTRACT

Respirometers have been designed, constructed and to a limited extent, tested to maintain and measure production and/or consumption of biogenic heat and carbon dioxide production and oxygen consumption for extended periods of time in approximately 0.5 l soil and/or litter microcosms. Using coniferous soil/litter microcosms, the mean coefficient of variation within sets of similar microcosms was 10.7% for the oxygen consumption rate and 3.9% for carbon dioxide production rate.

Microcosm respiratory response, population responses to moisture level where measured, succession, and salt effects were similar to those observed in the natural world.

Respiration of the decomposer communities in coniferous forest soil/litter microcosms was inhibited by treatment with "real world" salt concentrations of Cd, Se, Zn, Mn, Ni, Cu, Hg, Co, Cr, Va, Li, La, Ag, and Pb. These findings support the thesis that the consequence of these ecosystem disruptions might be to reduce primary and secondary production of the dependent populations. Scale drawings of the microcosm "life-support" system and an outline of procedural details of system maintenance and microcosm preparation are presented.

This report was submitted as partial fulfillment of inhouse research under Program Element 1AA006, ROAP 21 ALU, Task 3. It covers the period March, 1974, to November, 1976, and work was completed as of March, 1977.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Microcosms Trace Elements Heavy Metals Decomposition	Forest Soil/Litter	06/F

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